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DEVELOPING CONTROL STRATEGIES OF POTATO WART DISEASE (*SYNCHYTRIUM ENDOBIOTICUM*) IN TURKEY

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A B S T R A C T

The ultimate purpose of this study was to control Potato Wart Disease. Thus, during 2010 – 2011, in order to control the Potato Wart Disease caused by *Synchytrium endobioticum* the efficacy of seed fungicides and soil fumigants, the influence of 15 different plants used as alternative cropping to potato and the extracts derived from carrot, onion, garlic and radish were examined on the sporangium viability. The results revealed that the roots of turnip tuber were the most effective treatment among four different plant extracts used. Sunflower and Rye were found to be promising alternative crops to potato for the region with 74% and 73% of sporangium mortality, respectively. The most effective fumigant application on the viability of *Synchytrium endobioticum* sporangia in the soil was Formaldehyde with a 93.8 – 97.5 % efficacy rate. The fungicide efficacy on the diseases occurrence changed from 25.2 % to 62.5 % comparing to control plots. Although significant differences were obtained numerically between efficacy ratios, no statistically significant differences were found in all applications. The most effective application on Potato Wart Disease occurrence was the dosages of 60 and 80 g /m^2 of Metham Sodium among the four different concentrations of Formaldehyde, Metham Sodium, Dazomet and Urea as plant fertilizer.

Keywords: *Synchytrium endobioticum*, control, crop rotation, fumigant, plant extract, seed fungicide.

INTRODUCTION

Potato wart disease (PWD) is caused by the obligate soil-borne fungus called *Synchytrium endobioticum* (Schilberszky). Percival belongs to Chytridiomycetes class (Langerfeld, 1984). PWD was transported from the And Mountains, South America to the North America and Europe at the end of 19th century. Nowadays, PWD is widespread in all potato growing regions of Asia, Africa, America and Pacific Ocean countries (EPPO, 2004). It was first detected in Turkey in Ordu (Aybasti), Nigde (Agcasar) and Nevsehir (Kaymakli, Derinkuyu) provinces in 2004 (Anonymous, 2009).

Typical symptoms of PWD are the cauliflower type warts on potato tubers. In early stages, the warts vary in sizes from the pea to the hand punch within white to green coloration (Hampson, 1981). PWD can cause symptoms in all parts of potato plants below the soil surface, except roots (Hampson and Haard, 1980). The main sources of PWD spread are infected soil and infected plant materials (Hampson, 1995). The disease which usually prefers cool climates is currently known to exist in 43 countries (Baayen *et al*., 2006). The crop yield loss due to PWD varies between 50% and 100% of (Hampson, 1993; Melnik, 1998).

It has been reported that there is no chemical control the disease, yet. It is prevented with the application of strict quarantine measures, and it is essential that tuber and soil analyses be carried out to determine the fields infected with the disease. There is a zero tolerance disease. The field is considered to be infected in the incidence of even a single sport or a single diseased plant and potato farming is closed (EPPO, 1999).

Hampson and Coombes (1991) reported that the chemicals used for the eradication of pathogens that live in the soil for a long time are not preferred due to their phytotoxic and adverse environmental effects. Currently no practical method on control of the disease, but the growing resistant potato varieties is reported. PWD is also a very important disease which threatens not only

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potato growing areas but also all agricultural lands in the country. The presence of PWD, negatively affecting potato trade is listed as an external and domestic quarantine pest in Turkey.

The ultimate purpose of this study was to find out the efficacy of some applications to provide a basis for PWD control since there does not exist any control measure apart from quarantine legislations in Turkey. The study also aims to prevent the occurrence of the disease and to determine the opportunities to combat the disease with the chemical applications by identifying the factors that influence the zoospore secretion from sporangium.

In addition, the effects of some applications which could be the base of struggling against PWD having no applicable combat apart from quarantine measures were studied. The plant extracts applications which might cause by promoting and releasing the death of winter sporangium's zoospore were also tested since the plant extracts which can reduce the amount of inoculum in the soil and can be grown as an alternative to potato. Furthermore, the effects of chemicals on seeds and the effects of soil applications on the occurrence of the disease were investigated.

MATERIALS AND METHODS

The study design consisted of four trials to investigate the efficacy of seed fungicides and soil fumigants. The influence of 15 different plants used as alternative cropping to potato and the extracts derived from carrot, onion, garlic and radish were examined on the sporangium viability for the control of PWD caused by *Synchytrium endobioticum*. The results were compared to those of the control.

Preparation of *Synchytrium endobioticum* Inoculum and Compost Production: *S. endobioticum* inoculum and compost production were prepared using the fresh warts on potato tubers according to the method of Spieckermann and Kothoff, (1924). The warts were cut approx. 1cm. in size and added to sand by one portion of wart in three portions of sand (1W:3S). The mixture was incubated at 18-25°C in dark for six months and two replicates were conducted. The production of compost was completed in three steps: 1) one-third of the wart and the sand mixture in the clean trays were wetted with tap water and stirred every day during a period of two months. 2) wetting and stirring processes were carried out in weekly periods for two months 3) the compost was left to dry without any wetting and stirring process and kept at +4°C for further use.

The Efficacy of Plant Cuttings on the Amount of Sporangium: In this section, the parts of the winter vegetables such as onion, garlic, carrot, radish and potato plant sprouts, inoculum density were determined, and applied to the infected soil.. The application was then examined to find out how the plant extract was affected by the release of sporangium zoospores.

Samples were assessed according to the method suggested by Zelya and Menlik (1998) to determine the amount of sporangium in *Synchytrium endobioticum* infested soil. Afterwards, 500ml glass bottles were filled up with 400g soil and wetted with distilled water up to satisfied level. A total of 20 g obtained from potato shoots, green onion, green garlic, carrot and radish tubers with small cuts were added into each bottle and incubated in dark at 16ºC for 3, 6, 9 and 12 days intervals. Each character was repeated three times using randomized block design. Analysis of variance was applied to the data obtained, LSD multiple comparison test revealed the differences among the applications (Karman, 1971).

Efficacy of Alternative Plant Cultivation on the Amount of Sporangium: Fifteen cereals and winter crops alternative to potato were selected from the agricultural crops grown in the Turkish potato growing areas. The plants were Rye (Secale cereale), Sunflower (Helianthus annuus), Garlic (Allium sativum), Corn (Zea mays), Wheat (Triticum aestivum), Onion (Allium cepa), Alfalfa (Medicago sativa), Carrot (Daucus carota), Barley (Hordeum vulgare), Turnip (Raphanus sativus), Watermelon (Citrullus lanatus), Cauliflower (Brassica oleracea), Zucchini (Cucurbita pepo), Cabbage (Brassica oleracea), and Bean (Phaseolus vulgaris). Seeds from these fifteen crops were sown in pots with infected soil to test the capacity of their soil exudates to induce zoospore release from the sporangia in the soil. The initial density was determined as 35 sporangia/g soil. The pots were kept in the chambers provided with, 24 ° C and 70 % average humidity, 16 h light and 8 h darkness for 3 to 4 weeks then the amount of sporangium in the soil were counted (whether they are alive or not) to find out the sporangium death ratio and the efficacy of applications (Zelya and Menlik 1998). The experiment with four replications was set up on the randomly selected plots using randomized block design.

Effects of Seed Fungicide on PWD Occurrence: Fungicides from seven different active ingredients were used as seed treatment to observe the diseases

occurrence. The names and active ingredients were, Metalaxyl (200g/L) Aprin, Metalaxyl-M(40)+Acibenzolar-S-Methyl(%4) Bion, Azoxystrobin(75 g/L)+Fludioxoni (12.5 g/L)+Metalaxyl-M (37.5 g/L) Dynasty, Thiabendazole (%14) +İmazalil (%10) Fruitgard, Fludioxonil (25g/L)+ Metalaxyl-M Maxim XL, Tebuconazole(60g/L) Raxil and Pomarsol Forte (%80) Thiram. The study was conducted on infested soil in the pots (25 cm) in the open air. The pots were filled with the heavily infested soil obtained from the field with winter sporangium, and 150g inoculum from the pathogen compost was prepared (Spieckermann and Kothoff, 1924). The seedling of Marfona variety known as to be sensitive to PWD was sown in each pot after fungicide application. The dipping method was used in the fungicides application. The experiment was a completely randomized parcel design with 5 replications. After 120 days, the plants were pulled out and the wart occurrences on tubers were evaluated according to 1-9 scale (EPPO, 2004). Disease severities were calculated according to the scale values, and analysis of variance was applied to these values. Then, the LSD multiple comparison test was administered to determine the differences among the applications of fungicide seed treatments and the effects of Abbott applications on *S. endobioticum* were determined (Karman, 1971).

The Soil Fumigants and Urea Effects on PWD Occurrence: The formaldehyde, metham sodium and dazomet as fumigants and also urea as plant fertilizer were used on PWD control in the study. Treatment dosages were 20, 40, 60 and 80 g/m2 for dazomet, 20, 40, 60 and 80 ml/m2 for metham sodium, 100, 150, 200 and 250 ml/m2 for formaldehyde and 200, 400, 600 and 800 g/m2 for urea respectively. The dosages converted to the pots sized 25 cm in diameter were applied. The pots were filled with heavily *S. endobioticum* infested soil obtained from Derinkuyu (Nevsehir province), 150g of inoculum from the pathogen compost was prepared (Spieckermann and Kothoff, 1924) and the rest of pots were again filled with the infested soil. After watering the pots, they were covered with the polyethylene cover. After a week, the cover was removed for ventilation lasting two weeks. After the ventilation, half of the soil from each pot was transferred to new pots to saw Marfona seedling. The experiment with four replications was set up on the randomly selected plots.

After sowing, necessary maintenance and irrigation processes were performed periodically. After 120 days, the plants were pulled out and the wart occurrences on tubers were evaluated according to 1-9 scale (EPPO, 2004). Disease severities were calculated as mentioned above in (2.4).

RESULTS

The Efficacy of Plant Cuttings on the Amount of Sporangium: In this study, the effects of mixing the soil with the sprout of potato tuber, carrot-grassed radish tuber roots and leaf fragments diseased onion-garlic sporangium on the amount of sporangium were examined. The number of live sporangium, sporangium death rates and the effects rates of the applications in the soil samples examined at the end of the incubation were determined (Table 1).

Table 1. The Efficacy of Plant Cuttings on Winter Sporangia Viability of *S. endobioticum*.

Plant Material	3 Day		6 Day		9 Day		12 Day	
	Mortality ratio (%)	% Effect	Mortality ratio (%)	% Effect	Mortality ratio (%)	% Effect	Mortality ratio (%)	% Effect
Carrot	31.3 a*	30.4	31.3 a	25.8	30.7 b	26.7	36.0 c	22.0
Potato	26.7 a	25.6	30.0 a	24.4	30.0 b	26.0	48.7 bc	37.4
Garlic	40.0 a	39.1	34.0 a	28.7	41.3 ab	38.0	50.0 ab	39.0
Onion	30.0 a	29.0	24.0 ab	17.9	40.7 ab	37.3	48.0 bc	36.6
Turnip	30.0 a	29.0	38.7 a	33.7	55.3 a	52.9	63.3 a	55.4
Control	1.3 b	-	7.3 b	-	5.3 c	-	18.0 d	-

* Values with different letters are significantly different as LSD ($P < 0.05$).

In the control plots, where the soil was just wetted, the sporangium level slightly decreased to 41.0 sporangium/g soil due to natural death. The sporangium death rate was different from those of the control plots in all applications; yet no statistical difference was detected. The sporangium death rate in onion cuts application was different from those of the control plot although the results were found to have similar

statistical records on the 6th day. Soil applications consisting of radish, garlic and onion cuts on the 9th day, the sporangium death rate were recorded to be 55.3, 41.3 and 40.7%, respectively, which were the highest sporangium death rate in all the experiments conducted in the study. On the 12th day, the radish application displayed the highest sporangium death rate resulted in 63.3 %, and followed with 50.0% with the garlic application. The applications of these experiments resulted in the remarkable death of zoospores released from the sporangia. The most effective application of the study was recorded in the radish tuber.

Alternative Plant Cultivation Efficacy on the Amount of Sporangium: This study was conducted under the assumption that the root exudates spread during the germination of some alternative plant seeds which could be grown in the potato cultivation ecology were likely to reduce the amount of sporangium. It was observed that the plants having a high capacity to keep the soil dry were more effective as compared to the others.

Furthermore, the sunflower and rye among 15 different alternative plants were found to be having the highest sporangium death rate with 76.4 and 75.7% respectively. The sporangium death rate varied from 31.4 to 62.9% on the other alternative plants (Table 2).

Table 2. Effects on Viability of Winter Sporangia of Alternative Plant Root Exudates

Plants	Alive sporangium numbers	Sporangia mortality ratio (%)	(%) Effect
Rye	8.5	75.7 a*	73.0
Sunflower	8.3	76.4 a	73.8
Garlic	13.0	62.9 ab	58.7
Corn	13,5	61.4 ab	57.1
Wheat	16.0	54.3 ab	49.2
Onion	18.3	47.9 ab	42.1
Alfaalfa	18.3	47.9 ab	42.1
Carrot	18.5	47.1 ab	41.3
Barley	19.8	43.6 a-c	37.3
Turnip	19.8	43.6 a-c	37.3
Watermelon	21.5	38.6 bc	31.7
Cauliflower	23.0	34.3 bc	27.0
Zuccuni	24.0	31.4 bc	23.8
Cabbace	24.0	31.4 bc	23.8
Bean	23.8	32.1 bc	24.6
Control	31.5	10.0 c	-

* Values with different letters are significantly different as LSD (P < 0.05).

The beginning population of 35 sporangium/g soil was given to the control wetted soil pots and the death rate from natural causes was found as 10%.

The alternative plants efficacy varied from 23.8% to 73.8% according to the natural sporangium death in control pots (Table 2). Among the alternative plants of maize, garlic, wheat, onion and alfalfa, the death rate of sporangium was found as 50%, and this rate was considered to be promising for future experiments. The rest of the vegetables, such as beans, zucchini, cauliflower and cabbage cultivated in Nigde and Nevsehir provinces showed lower efficacies with 25%.

Effects of Seed Fungicide on the Diseases Occurrence: Among the disease occurrence and efficacy of seed fungicides in all applications conducted on the potato variety, Marfona always showed lower rate than those of the control plot in terms of the virulence (Table 3).

However, no statistically significant differences were found among the fungicides of Bion, Fruitgard, Maxim and Thiram. The most effective application (9.6%) was obtained from Aprin (active ingredient; Metalaxyl). Apart from control plot and Maxim, the results of other fungicides were statistically close to the efficacy of Aprin (Table 3). The disease occurrence varied from 25.2% to 62.5 % among the applications of fungicides when compared to those of the controls, yet no statistical differences occurred among all the applications.

Table 3. Effects on Disease Severity of *S. endobioticum* of Seed Fungicides.

Fungicides	Diseases Severity (%)	(%) Effect
Aprin	9.6 a*	62.5 a
Bion	16.9 a-c	34.0 a
Dynasty	12.2 ab	52.2 a
Fruitgard	17.0 a-c	33.4 a
Maxim	19.2 bc	25.1 a
Raxil	12.1 ab	52.8 a
Thiram	16.9 a-c	34.0 a
Control	25.6 c	-

* Values with different letters are significantly different as LSD (P < 0.05).

The Soil Fumigants and Urea Effects on the Diseases Occurrence: In order to determine the disease occurrence, the fumigants applications and urea were studied. The disease severity and efficacy ratio were determined, accordingly (Table 4).

The dosages of metham sodium at 60 and 80 g /m2 were found to be the most effective. This was followed by 40 g of metham sodium (11.1%), four dosages of formaldehyde (11.1-11.8%), 200-600 g of urea (11.1-12%) and 40 g of dazomet (11.7%) dosages. The disease virulence was observed to be 8.3% in the application plot, as it was only 29% in the control. The highest disease virulence after control was recorded as 17.7% on the 60 g of dazomet dosage.

Table 4. Effects on Disease Severity of *S. endobioticum* of Soil Fumigants.

Application rates (g-ml/m^2)	Diseases severity (%)	(%) Effect
Dazomet 20	12.7 a-c*	56.2 a-c
Dazomet 40	11.7 ab	59.5 ab
Dazomet 60	17.7 c	38.7 c
Dazomet 80	15.4 bc	47.0 bc
Formaldehide 100	11.8 ab	59.0 ab
Formaldehide 150	11.1 ab	61.6 ab
Formaldehide 200	11.1 ab	61.6 ab
Formaldehide 250	11.1 ab	61.6 ab
Metham sodium 20	15.2 bc	46.5 bc
Metham sodium 40	11.1 ab	61.6 ab
Metham sodium 60	8.3 a	70.9 a
Metham sodium 80	8.3 a	70.9 a
Urea 200	11.1 ab	60.7 ab
Urea 400	11.9 ab	58.9 a-c
Urea 600	12.0 ab	58.4 a-c
Urea 800	14.0 bc	51.5 a-c
Control	29.0 d	-

* Values with different letters are significantly different as LSD ($P < 0.05$).

DISCUSSION

In a study, to investigate the role of alternative plants and the impact of root exudates on sporangium amount were designate. In the present study, the interaction between the sporangium amount and root exudates released during the germination of seeds was taken into consideration. The highest sporangia death rate was obtained from the sunflower and rye among the 15 different plants alternative to potato cultivation. The crops such as cereals, maize, garlic, onion and alfalfa whit could keep the cultivation soil as dried in the areas infected with PWD were found to be significantly efficient on the rate of sporangia death (Gunacti and Erkilic, 2010).

Hampson (1979), demonstrated that leaving the diseases infected field to bare fallowing for 5 years subsequent to the cultivation of the tolerant variety, zucchini, maize, fava beans suppressed the PWD in Belarus. The same researcher suggested that leaving the infected field to fallow for about 3 to 5 years could result in 97% zoospore germination. The zoospore germination rate in this study increased up to 75% by using organic fertilizers derived from poultry, pig and cow.

The application of winter vegetable cut into the wetted soil in the areas where potato could be cultured resulted in the remarkable amount of zoospores released from sporangia following their death, and radish tuber application was found to be the most effective one out of 15 varieties of winter vegetables.

There have been many studies on the effects of plant extracts and composes on plant root and leaf pathogens. The results revealed that high percentages up to 100 % were affected while some of the pathogens were either triggered or not affected (Thompson, 1986; Stind and Weltzien, 1988; Çakır and Yeğen, 1991). King and Smith (1968) maintained that the compound gained from the onion and garlic extracts would inhibit the sclerots of *Sclerotinia cepivorum*'s and that the garlic extracts were better inhibitors than onion extracts. When the root and tuber extracts of such plants used in the study as radish, onion and garlic were blended in the contaminated soil, and then when the effects of this process on sporangium in the soil and the seeds of the same plants sown in the same soil were compared, it was observed that the plant extract applications were more effective than seed applications. This was assumed to have been resulted from the chemical compounds in plant extracts (Gunacti and Erkilic, 2010).

Systemic fungicides are reported to be the least effective ones against Oomycetes (Hampson, 1977). In this study, the fungicides were assumed to have a compound value in respect to PWD. In addition, Erwin (1973) stated that a difficulty with chemical control of PWD might lead to the infection court. Erwin also maintained that systemic fungicides could hardly effect the Oomycetes fungicides due to the difficulty to combat the disease with PWD in infected fields, yet some of the components seemed to be encouraging. Comparison between control and fungicides application and the diseases occurrence varied between 25.2 and 62.5 %. However, the efficacy rates of figures were different from each other, though

there were no statistically significant differences between all applications.

In addition, with a zero tolerance of *S. endobioticum,* in gram soil even only one sporangium can cause infection (Hampson, 1993; EPPO, 2004). In the study of Hampson (1992), it was recorded that 0.25 sporangium in gram soil was able to initiate infection. However, the number of zoospore releasing sporangium was reported to be correlated more or less with the number of warts and their sizes, as well (Zakopal and Spitzova, 1959; Hampson, 1992; Browning 1995).

In another study, Hampson (1977) recorded 9 fungicides against the strain no. 2 of *S. endobioticum* used by dipping the tubers into the fungicide solutions, yet none of them were able to control the PWD in the field stage.

Up to date, among more than 400 experiments, no compound has been reported to control PWD, with high efficiency and adequate cost to apply in practice (Crowther *et al.,* 1927; Gimingham, 1919; Hartman, 1955; Roach, 1925; Zakopal, 1950; Olsen, 1966; Kharitonova, 1969; Hampson, 1977; Hampson, 1993).

To check the efficacy of soil fumigants and urea on disease occurrence, a study was carried out. After testing the four different concentrations of each chemical, the most effective application was reported to be metham sodium in the dosages of 60 and 80 g /m2 with 70.9 % efficacy. In the control plot, a decrease up to 29 % was observed on PWD virulence while it was only 8.3% in the application plot. Similarly increasing the fungicide dosages to the for tubers were resulted in no significant decrease on diseases virulence in this study. This result was associated with the behavior of the PWD sporangium and zoospore. The variance was, therefore, found to be quite high in the replicates of the experiments. This might have been resulted from two reasons; the distributions of pathogen sporangium's were homogeneous and the zoospore releases at different times (Stenz, 1963; Hampson, 1981; Hampson *et al*., 1994).

The results related with the soil fumigants efficacy on PWD obtained in this study are associated with those of the related studies. Potocek (1991) reported that the dosage of 100 ml/m2 metham sodium was successful in controlling the disease and dazomet at 60 gr/m2 dosage were also found effective. Although there hasn't been any current study on formaldehyde as a soil fumigant, Roach and Glynne (1928) highlighted the success of formaldehyde to control PWD. In addition, Reed and Dickens (1993) suggested that the disinfection machinery, equipment by formaldehyde was the most effective way to prevent the disease spread.

Methyl bromide has been used in intensive agricultural land for soil disinfection for a long time. In some studies, methyl bromide has also been tested on *S. endobioticum*, which has resulted in success (Noehr and Mygind, 1977; Pearson *et al.,* 2007). Similarly, chloropicrin, one of the active ingredients in methyl bromide, also has fungicidal impact as soil fumigant alone. Kharitonova (1969) tested and suggested it against PWD control.

Among the studies to control PWD by nitrogen fertilizers, urea and ammonium nitrate can be cited as the most studied ones. Potocek (1983), pointed out that the urea dosages of 1-1.5 kg/ m2 could be an effective disease control. However, in the current study, 200g dosage usage resulted in an efficacy rate of 60,7 %, which is promising. The differences on dosages and efficacy of nitrogen fertilizers reported in various studies might be resulted from the differences between the soil types and the strains of the pathogen (Hampson and Coombes, 1996). The differentiations among the results obtained in previous experiments might be associated with the presence of 43 strains of *S. endobioticum* (Baayen *et al.*, 2006). The efficacy of urea on pathogen control was not achieved because of its chemical structure. It was determined according to the result of the pH changes in the soil (Weiss, 1925; McLaren and Peterson 1967; Hampson, 1980). Weiss (1925) reported that infection by pathogen could occur at a pH level of 3.9-8.5. Furthermore, Zakopal and Spitzova (1959) suggested that PWD could be developed at a pH level of 2.5-12.38. The optimum growth of the pathogen for infection was also considered between the pH levels of 6-8. However, these results conflict with the results of Hampson (1985) on the mode of urea action. Hampson (1985) claimed that the impact of urea in the soil pH level has an important on the efficacy of diseases occurrence that was claimed because of acidic character of urea with a decreasing affect in the soil pH level. The soil pH values of this study were between 7.55–7,58. To control PWD, it is not feasible to decrease the soil pH below 2.5 and 3.9 which triggers the pathogen infection by applying high amount of urea. In addition, using a high amount of urea to combat disease is not likely to reduce the soil pH below 2.5-3.9 levels which initiates pathogen infection.

Even if it was achieved, it would be too acidic to cultivate

potato crop under the indicated pH, hence, more detailed studies should be carried out on the pH intervals for pathogen infections and mechanism of urea action on disease occurrence in order to reveal much more reliable results. In such situations, urea and other similar chemicals naturally could show different effects in different soil textures.

In conclusion, considering the characteristic feature of the disease, and the zero tolerance of the pathogen in soil, we maintain that any practice alone to control PWD is not sufficient. Removal of the soil and tuber should be strictly avoided. Two major problems could be encountered in chemical control of PWD; the chemicals cited as effective against PWD pose a threat to human, animal and plant health and controlling the disease with the application of integrated product management is limited. Therefore, the main principle in PWD control should be achieved only by applying strict quarantine measures to prevent the disease spread.

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