



Epidemiological Studies, viz. Effect Of Soil Temperature, Moisture, Electrical Conductivity (EC), Soil pH on *Sclerotium rolfii* Sacc. Causing Sclerotium Wilt or Rot in Potato and Its Survival and Host Range

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ABSTRACT

Sclerotium wilt/ rot of potato caused by Sclerotium rolfii Sacc. is one of the major soil borne diseases of potato causing heavy losses every year. To know the epidemiology and to develop suitable management practice the study was carried out. The results revealed that, Four per cent inoculum level was necessary to get 100 per cent infection. The maximum per cent germination of sclerotia (87.50%) was noticed at 1 cm depth, which gradually reduced with increase in depth. The germination of sclerotia was 100 per cent upto one month after the storage and it gradually decreased with increase in storage duration.

*The rate of movement of mycelia at low inoculum level (one sclerotia) started its movement very slowly and took eight days to reach maximum distance of 9 cm, whereas, the rate of movement was visible on 2nd day itself in high inoculum (five sclerotia) and reached maximum of 9 cm distance on 6th day only. Colonization of sorghum seeds and germination of sclerotia were drastically reduced with increase in EC levels of soil. The fungus made maximum growth and germination of sclerotia at 30° C. The optimum soil temperature for the growth of *S. rolfii* and germination of sclerotia was found between 25-30° C.*

The maximum saprophytic activity of the fungus and germination of sclerotia were found at 30 per cent soil moisture level, the fungus showed moderate to good growth over a pH range of 5.5 to 9.0. Maximum fungus colonization on sorghum baits was recorded at pH 6. Highest germination of sclerotia was observed at pH of 5.5.

Keywords: *Sclerotium rolfii*, Sclerotia, Soil moisture, Soil temperature, pH

INTRODUCTION

The potato (*Solanum tuberosum* L.) plant is a member of solanaceae or the nightshade family. Potato is one of the important and widely grown vegetables of the world, introduced in 17th century. The mineral

production in case of potato is 3.70 times more than wheat and 11 times more than rice. Potato gives more carbohydrates, fiber and vitamins per unit area and time than the other major food crops.

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Potato is low energy food, 200g of boiled potatoes provide about 138 Kcal of energy (Shekhawat & Dahiya, 2000). It is rich in potassium and phosphorus (Shekhawat et al., 1992). Tubers contain at least 12 essential vitamins and is a good source of vitamin 'C' containing about 14-25 mg/10g of fresh weight of tubers (Thornaton & Siczka, 1980).

Potato wilt caused by *Sclerotium rolfsii* Sacc. is a well known polyphagous, ubiquitous and a non-target pathogen. It is one of the most destructive soil inhabiting pathogens so far reported. It has attained a serious status in Northern Karnataka particularly in the transitional belt. It is essential to develop a suitable Integrated Disease Management (IDM) with cultural practices, organic amendments and biological management practices. In order to reduce the environmental hazards, to avoid the development of resistant strains and reduce the cost of cultivation.

MATERIALS AND METHODS

Inoculum levels and the disease incidence

Local (UASD) isolate was used for all epidemiological studies. The soil, sand and farmyard manure were sieved by passing through 2 mm mesh and sterilized separately and then mixed these material in 1:1:10.5 per cent proportion. After mixing, it was weighed and filled in surface sterilized earthen pots. *S. rolfsii* culture grown on sand corn meal medium for 30 days (containing mycelium and sclerotial bodies) was mixed separately to each pot to obtain different inoculum levels viz. 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 per cent and each treatment was replicated thrice. The pot filled with sterilized soil without any inoculum served as control (un-inoculated). Apparently healthy potato tubers of variety Kufri Chandramukhi were surface sterilized with 1:1000 mercuric chloride solution for 90 sec. then washed thrice in tap water and were sown in pots. Three replications were maintained for each treatment. Water was added to the pots at regular intervals to maintain 25-30 per cent soil moisture. The observations were recorded on 7th day for pre-emergence death and 20th

day for post-emergence death of seedlings, which was used for calculating the per cent infection.

Viability of sclerota of *Sclerotium rolfsii* at different depths and duration in Soil

This study was conducted to get the information on the survival of *S. rolfsii* at different depths of soil and for different durations of time. Sclerotia of the *S. rolfsii* was obtained from giant culture. Approximately 100 sclerotia were wrapped with small quantity of soil in nylon net (mesh size 1mm) and 3 such bundles were buried at different depths ranging from 1 to 20 cm with difference of one cm, for 12 months in surface sterilized earthen pots (30 cm diameter) containing field soil after the exposure of 12 months, all the nylon bundles of sclerotia were taken out and the Sclerotial viability was tested (Mishra et al., 1995 & Gurjar et al., 2004). The bundles were also buried for different durations, in each of the 20 surface sterilized earthen pots (15 cm diameter) at depth of two cm. The pots were kept in the glass house. To test the duration of viability of sclerotia, one bundle each from 20 pots were taken out at monthly interval by dipping in 0.1 per cent mercuric chloride solution for one minute followed by three washings with sterilized distilled water and inoculated aseptically to petriplates containing PDA by following the standard procedure. The per cent germination of sclerotial bodies was recorded.

Rate of movement of *Sclerotium rolfsii* in soil with different levels of inoculum

To know the rate of movement, the field soil was sterilized and placed in a 20 cm (200 mm diameter) petriplates. One, two and five sclerotial bodies were kept at the center. The previously sterilized sorghum seeds were kept around the sclerotia at an equal distance of one cm. The optimum moisture was maintained. Observations were made at every 24 hrs to measure the distance traveled by the mycelia of sclerotia.

Effect of soil electrical conductivity (EC)

To know the favourable EC for growth of *S. rolfsii* the experiment was carried out. The soil sample was collected from fields having EC

readings, viz. 0.4, 3.4, 6.9, 8.9 and 10.00 ds/m, lowest EC was taken as control. Such soils of different EC were sieved through sieve and mixed with giant culture at the rate of four per cent of soil and filled in 20 cm (200 mm diameter) Petriplates. Each treatment was replicated 4 times. The plates were kept in incubators for seven days, with 25 per cent moisture and 30°C temperature. After seven days the plates were taken out and sterilized healthy sorghum seeds, were placed equidistantly on it at the rate of ten seeds per plate. Per cent colonization on sorghum seeds was determined. One hundred sclerotia of *S. rolfsii* were kept in each treatment to know the viability of sclerotia. After seven days of incubation sclerotial bodies were plated on PDA by following standard procedure and per cent germination of sclerotial bodies were recorded.

Effect of soil temperature

This experiment was conducted to know the optimum temperature required for growth and development of the pathogen. Per cent colonization on sorghum seeds at different soil temperatures, viz. 10°, 20°, 30°, 40° and 50°C was tested and germination of sclerotia was also recorded.

Finely powdered, sterilized soil sieved through two mm sieve was mixed with the giant culture at the rate of four g per 100g of soil. Each Petriplate was filled with 100 g of soil infected with the culture. The soil in the study was moistened to 25 per cent moisture holding capacity (MHC) by adding required quantity of water every day. Each treatment was replicated thrice. The plates were incubated in incubator to the required temperature levels for seven days.

After seven days, the plates were taken out and sterilized healthy sorghum seed, were placed at equidistance on it at the rate of ten seeds per plate. On the 4th day observations were recorded on per cent colonization of *S. rolfsii* on sorghum seeds. One hundred sclerotia of *S. rolfsii* were kept in each temperature to know the viability of sclerotia in each treatment. After seven days of incubation sclerotial bodies were plated on

PDA by following standard procedure and per cent germination of sclerotial bodies were recorded at each temperature.

Effect of soil moisture

This experiment was conducted to know the favourable moisture conditions for of pathogen. Autoclaved soil was mixed with giant culture so as to make four per cent giant culture. The Petriplates of 20 cm diameter were filled with the inoculum. The soil moisture levels were adjusted to 10, 20, 30, 40, 50, 60 and 70 per cent of moisture holding capacity and the moisture level in each treatment was maintained throughout experiment by adding water to nullify the evaporation loss. Each treatment was replicated thrice. After seven days of incubation, the survival of the fungus was determined by baiting technique and the percent colonization of the fungus on sorghum seeds was calculated. One hundred sclerotia of *S. rolfsii* were kept in each treatment to know the viability of sclerotia. After seven days of incubation, sclerotial bodies were plated in PDA by following standard procedure and per cent germination of sclerotial bodies were recorded.

Effect of soil pH

This experiment was conducted to know the exact pH range for growth and development of the *S. rolfsii* in the soil. Twenty gram of soil was taken and suspended in 40 ml sterile distilled water in 250 ml conical flask and stirred for 30 min. to get the soil to water ratio of 1:2, pH of this soil was 7.5. The soil pH was adjusted to 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0. and 9.5 by using Na₂CO₃ and Oxalic acid. Hundred grams of soil of each pH level was taken and 4g of giant culture was mixed to each treatment and placed in 20 cm diameter petriplates. Each treatment was replicated thrice. The soil in the study was maintained at 25 per cent moisture holding capacity (MHC) by adding required quantity of water every day. The plates were incubated at 30°C for seven days. After seven days healthy sorghum seeds, previously boiled in water, were placed equidistantly on it at the rate of 10 seeds per plate. Per cent colonization on sorghum seeds

was recorded. A separate 100 sclerotia of *S. rolfsii* were kept in each treatment to know the viability. After seven days of incubation, sclerotial bodies were plated on PDA by following standard procedure and per cent germination of sclerotial bodies were recorded.

RESULT AND DISCUSSION

Inoculum levels and disease incidence

The inoculum was mixed to the soil on w/w basis at different levels viz., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 per cent as described in 'Material and Methods'. The potato tubers of variety Kufri Chandramukhi were used for planting in the pots. The pre-emergence, post emergence and total disease incidence were recorded (Table 1).

The results indicated that there was cent per cent pre-emergence disease incidence in 8 per cent inoculum levels and above i.e no pre and post emergence disease incidence was noticed in uninoculated condition. Minimum pre-emergence disease incidence (31.67%) was noticed in one per cent inoculum level and increased with increase of inoculum and reached 100 per cent at eight per cent inoculum level which was on par with four (91.67%), five (95.00%), six and seven per cent (98.33%).

Minimum post emergence disease incidence (11.67%) was noticed in one per cent inoculum level and maximum (18.33%) was recorded in two and three per cent inoculum level. But in subsequent higher inoculum levels, less per cent disease incidence was noticed as majority of plants were killed because of pre-emergence death. With respect to the total incidence, 43.34 per cent disease was observed at one per cent inoculum level and increased to 83.33 per cent at two per cent, 93.33 per cent at three per cent and 100 per cent at four per cent inoculum level. Hence, 4 per cent inoculum level was used in further studies. Previously, Prabhu (2003) recorded a similar trend of disease incidence in soybean with increase in inoculum levels, and 100 per cent disease incidence was noticed in and above four per cent. At inoculum level of eight per cent, 100

per cent pre emergence death of the tubers was noticed. This trend clearly showed that, as there was increase in inoculum level, then mortality of the seedlings was also high. Though the inoculum levels of 1, 2 and 3 per cent recorded lesser per cent of pre emergence mortality, there was considerably higher per cent of post emergence death of the seedlings. Higher inoculum density resulted in cent per cent pre-emergence mortality, which may be due to the fact that higher inoculum always ensures the certainty of the infection. Similar results have been reported by Nargund (1981), Palakshappa et al. (1987), Harlapur, (1988), Hanumanthegouda (1999) and Prabhu (2003) also reported 100 per cent pre-emergence incidence at inoculum density levels of four per cent and above in wheat, betel vine, wheat, groundnut and soybean crops respectively.

Viability of sclerotia of *S. rolfsii* at different depths and duration in soil

An experiment was carried out in the glass house of Department of Plant Pathology, College of Agriculture, Dharwad to know the viability of sclerotia at different depths and duration as described in 'Material and Methods'. The per cent germination of sclerotia was recorded at different time intervals and specific depths (Table 2).

The experimental results indicated that maximum per cent germination of sclerotia (87.50%) was noticed at one cm depth which was significantly superior over all other treatments. Per cent germination was reduced gradually as the depth increased. Significantly least germination of 10 per cent was noticed at 19 and 20 cm depths.

The germination of sclerotia was 100 per cent at the beginning of the storage and one month after the storage which was significantly superior than all the treatments and it was gradually decreased with increase in storage duration. The germination was least (12.50%) at the end of 19th month after storage, but on par with 17 (17.50%) and 18 months (15.00%) of storage. Gurjar et al. (2004) reported that, *S. rolfsii* lost its viability after 18 months of burial and Mustafee and Chattopadhyay (1983) reported loss of

viability after 17 months. In the present study also similar trend was observed.

Rate of mycelial movement of *S. rolfsii* in soil with different levels of inoculum

A laboratory experiment was conducted to know the movement of mycelia from sclerotia in the soil over time. The observations on movement of mycelial growth were measured at 24 h intervals and data were analysed statistically and presented in table 3.

The results indicated that there was no movement of hyphae after one day with a single sclerotial body, but there was movement of hyphae of 1 cm and 0.50 cm where five sclerotia and three sclerotia were kept respectively. The hyphal movement was started in one sclerotial body (0.50 cm) on third day. In plate with five sclerotia the hyphal movement was covered 9 cm on 6th day, but in plates with one sclerotial body and three sclerotia the movement of hyphae took eight and seven days respectively. Punja and Gragan (1981) observed that, the infection of the host tissue by dried sclerotia was greatly increased, when infection was from three cm distance in quartz sand and three cm in the field soil.

Effect of soil electrical conductivity (EC)

Five levels of electrical conductivity viz., 0.4, 3.4, 6.9, 8.9 and 10 dS/m were tested against *S. rolfsii*. In general, the fungus survived better at lower EC levels than at higher EC levels of 8.9 and 10 dS/m. The maximum saprophytic activity of the fungus was 90.00 per cent at EC level of 0.4 dS/m. The activity of the fungus decreased as EC levels increased from 0.4 to 8.9 dS/m and at EC level of 10.0 dS/m, the activity of the fungus was completely ceased (0). The effect was also seen on per cent germination of sclerotia, which was 100 per cent at 0.4 dS/m EC level which was significantly more compared to all EC levels. The germination decreased to 70 per cent at 3.4 dS/m EC, 33.75 per cent at 6.9 dS/m and 31.25 per cent at 8.9 dS/m EC level. Minimum germination of sclerotia (6.25 %) was observed in 10.00 dS/m EC level which was significantly less than all other EC levels tested (Table 4).

This is in conformity with the work of Palakshappa (1986) and Harlapur (1988) in case of *Sclerotium rolfsii* of betelvine and wheat respectively. Higgins (1927) reported that, the fungus growth was decreased by adding sodium chloride into the medium, thereby indicating the toxic nature of the salt. Lingaraju (1977) reported, no activity of *S. rolfsii* at the EC levels of 6.9 to 8.9 dS/m. In the present studies also same results were obtained.

Effect of soil temperature

Soil temperature is one of the important factors influencing the growth of the fungus. The soil temperatures tested were viz., 15, 20, 25, 30, 35, 40, 45 and 50° C as described in 'Material and Methods'. The per cent colonization of sorghum seed baits at different soil temperatures and the germination of sclerotia are given in table 5.

The fungus made maximum growth at 30° C (60.00% colonization) which was significantly superior to all levels of temperatures tested. The next best temperature was 25° C in which the saprophytic activity of the fungus was 53.33 per cent. This indicates that, the optimum soil temperature for the growth of *S. rolfsii* would be between 25-30° C. However, there was no significant difference between the temperatures of 20° C and 35° C. No growth was noticed at 50° C. Similar trend was observed in per cent germination of sclerotia. Maximum germination of 90 per cent was observed at 30°C which was on par with 25° C (75.00%), and were significantly superior to all other treatments. It shows that, the temperature between 25-30° C is best for germination of sclerotia also. The reduced activity of the fungus at higher temperatures may be due to the lack of proper supply of oxygen needed for the optimum growth of fungus. This is in agreement with the findings of Manjappa (1979) and Harlapur (1988) in case of *S. rolfsii*.

Effect of soil moisture

The effect of seven soil moisture levels were tested on saprophytic activity of the fungus and the germination of sclerotia. The data

were analysed statistically and are presented in table 6.

The results revealed that, *S. rolfsii* survived better at low soil moisture levels than at high moisture levels. The saprophytic activity of the fungus was 30.00 per cent at 10 per cent soil moisture level and increased to 56.67 per cent at 20 per cent soil moisture level. The maximum saprophytic activity of the fungus was found at 30 per cent soil moisture level (83.33%), which was on par with 40 per cent (66.67%). Least saprophytic activity was recorded at 70 per cent soil moisture level (13.33%). Maximum of 100.00 per cent germination of sclerotia was observed at 30 per cent moisture level which was significantly superior over all other treatments. This was followed by 20 per cent (78.33%) which was on par with 10 per cent (70.00%) and 40 per cent (76.67%). Least germination of sclerotia was noticed in 70 per cent moisture level (36.67%) (Plate 9). This is in agreement with the findings of Manjappa (1979) and Harlapur (1988) in case of *S. rolfsii*.

Effect of soil pH levels

The fungus showed moderate to good growth over a pH range of 5.5 to 9.5. However, maximum fungal colonization of sorghum

seeds was recorded at pH 6, where the saprophytic activity of the fungus was 76.67 per cent which was on par with 6.5, 5.5, 8.0 and 9.0 pH (70, 60, 60 and 56.67%, respectively). Lower saprophytic activity of 46.67 per cent was observed at pH of 9.5 (Table 7).

Treatments differed significantly with respect to per cent germination of sclerotia also. Highest germination of sclerotia was observed at pH 6.5 (90%) which was on par with 5.5 (88.33%), 6.0 (85.00%), 7.0 (83.33%) and 7.5 (81.67%) pH levels. Significantly least germination of sclerotia was noticed at pH level of 9.5 (58.33%) which was on par with 8.5 and 9.0 pH (both 63.33%) levels.

Gondo (1962) reported that, the fungus growth was good at pH level 5.6 and he also concluded that the fungus growth was better in non-sterile soil at all pH levels. Choudhury (1946) reported that there was no correlation between soil pH and the disease incidence in case of betel vine. The present findings are in accordance with the observations of Palakshappa (1986) and Harlapur (1988) in case of *S. rolfsii* on betelvine and wheat respectively. Hence any little difference in soil pH will not affect the incidence of the disease.

Table 1: Effect of inoculum levels on incidence of potato Sclerotium wilt of potato

Inoculum levels (%)	Disease incidence (%)				Total
	Pre-emergence		Post-emergence		
0	0.00	(0.00)*	0.00	(0.00)	0.00
1	31.67	(34.23)	11.67	(19.89)	43.34
2	65.00	(54.02)	18.33	(25.30)	83.33
3	75.00	(60.08)	18.33	(25.31)	93.33
4	91.67	(73.40)	8.33	(16.60)	100.00
5	95.00	(77.08)	5.00	(12.92)	100.00
6	98.33	(85.70)	1.67	(4.31)	100.00
7	98.33	(85.69)	1.67	(10.46)	100.00
8	100.00	(90.00)	00.00	(00.00)	100.00
9	100.00	(90.00)	00.00	(00.00)	100.00
10	100.00	(90.00)	00.00	(00.00)	100.00
S.Em±	2.54		2.32		
CD at 1%	10.24		9.33		

* Figures in parenthesis are arc sin transformed values

Table 2: Viability of sclerotia of *Sclerotium rolfii* of potato at different depths and duration in the soil

Depth (cm)	Germination of sclerotia (%)		Duration (months)	Germination of sclerotia (%)	
1	87.50	(69.39)*	0	100.00	(90.00)
2	77.50	(61.72)	1	100.00	(90.00)
3	80.00	(63.23)	2	95.00	(77.08)
4	77.50	(61.72)	3	87.50	(69.39)
5	72.50	(58.39)	4	82.50	(65.32)
6	67.50	(55.26)	5	80.00	(63.61)
7	70.00	(56.79)	6	75.00	(60.11)
8	67.50	(55.26)	7	75.00	(60.00)
9	55.00	(47.88)	8	72.50	(58.40)
10	57.50	(49.32)	9	72.50	(58.40)
11	55.00	(47.88)	10	70.00	(56.79)
12	50.00	(45.00)	11	67.50	(55.26)
13	42.50	(40.68)	12	57.50	(49.36)
14	40.00	(39.23)	13	52.50	(46.44)
15	32.50	(34.74)	14	50.00	(45.00)
16	25.00	(29.89)	15	45.00	(42.11)
17	25.00	(30.00)	16	35.00	(36.22)
18	22.50	(28.28)	17	17.50	(24.68)
19	10.00	(18.44)	18	15.00	(22.79)
20	10.00	(18.44)	19	12.50	(20.61)
S.Em±	1.50			2.00	
CD at 1%	6.10			8.10	

* Figures in parenthesis are arc sin transformed values

Table 3: Rate of movement of *Sclerotium rolfii* of potato in the soil with different levels of inoculum over a period of time

Days	Average distance moved (cm)		
	One sclerotium	Three sclerotia	Five sclerotia
1	0.00	0.00	0.00
2	0.00	0.50	1.00
3	0.50	1.67	2.67
4	1.67	2.33	3.33
5	3.33	4.33	6.67
6	5.33	6.67	9.00
7	8.00	9.00	9.00
8	9.00	9.00	9.00
S.Em±	0.26	0.30	0.34
CD at 1%	1.11	1.29	1.44

Table 4: Influence of soil EC on competitive saprophytic ability and viability of *Sclerotium rolfii* of potato

Soil EC level (dS/m)	Per cent colonization of sorghum seeds		Per cent germination of sclerotia	
0.4	90.00	(71.57)*	100.00	(90.00)
3.4	37.50	(37.66)	70.00	(56.83)
6.9	22.50	(28.22)	33.75	(35.48)
8.9	6.25	(12.44)	31.25	(33.82)
10.0	0.00	(0.00)	6.25	(14.30)
S.Em±	2.34		1.74	
CD at 1%	10.13		7.54	

* Figures in parenthesis are arc sin transformed values

Table 5: Influence of soil temperatures on competitive saprophytic ability and viability of *Sclerotium rolfii* of potato

Soil temperature (°C)	Per cent colonization of sorghum seeds		Per cent germination of sclerotia	
15	20.00	(26.57)*	35.00	(27.18)
20	26.67	(30.99)	43.00	(30.87)
25	53.33	(46.42)	75.00	(45.06)
30	60.00	(54.85)	90.00	(53.96)
35	39.33	(38.23)	68.67	(41.49)
40	23.33	(28.78)	58.33	(37.37)
45	11.67	(19.85)	28.33	(23.81)
50	0.00	(0.00)	15.00	(16.95)
S.Em±	1.92		2.80	
CD at 1%	8.08		11.78	

* Figures in parenthesis are arc sin transformed values

Table 6: Influence of soil moisture on competitive saprophytic ability and viability of *Sclerotium rolfii* of potato

Per cent soil moisture	Per cent colonization of sorghum seeds		Per cent germination of sclerotia	
10	30.00	(33.00)*	70.00	(42.75)
20	56.67	(48.85)	78.33	(46.72)
30	83.33	(66.64)	100.00	(90.00)
40	66.67	(54.78)	76.67	(45.86)
50	50.00	(45.00)	60.00	(38.09)
60	23.33	(28.29)	55.00	(35.91)
70	13.33	(21.15)	36.67	(27.90)
S.Em±	3.56		2.12	
CD at 1%	15.39		9.18	

* Figures in parenthesis are arc sin transformed values

Table 7: Effect of soil pH on competitive saprophytic ability and viability of *Sclerotium rolfii* of potato

Soil pH	Per cent colonization of sorghum seeds		Per cent germination of sclerotia	
5.5	60.00	(50.85)*	88.33	(70.17)
6.0	76.67	(61.22)	85.00	(67.40)
6.5	70.00	(57.00)	90.00	(71.57)
7.0	53.33	(46.92)	83.33	(65.95)
7.5	53.33	(46.92)	81.67	(64.69)
8.0	60.00	(50.85)	78.33	(62.29)
8.5	53.00	(47.00)	63.33	(52.86)
9.0	56.67	(48.85)	63.33	(52.78)
9.5	46.67	(43.08)	58.33	(49.80)
S.Em±	3.54		1.75	
CD at 1%	14.60		7.25	

* Figures in parenthesis are arc sin transformed values

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