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Development of Suitable Package Using Bio-fertilizers For Management of Late Blight of Potato Under Climate Change

Morajdhwaj Singh, S.K. Biswas*, Kishan Lal, Devesh Nagar, Jaskaran Singh and Prem Naresh

Department of Plant Pathology, Chandra Sekhar Azad University of Agriculture & Technology, Kanpur - 208 002, India.

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Among the different packages of bio-fertilizers, soil application of mustard cake + tuber treatment + foliar spray with *T. viride* was found best in stimulating germination and increase plant height of potato at different days after sowing, representing the value of emergence of seedling at 12 days after sowing against 21 days in case of control. Similarly, plant height of potato was also found maximum in treatment T_7 (soil application of mustard cake + tuber treatment and foliar spray with *T. viride*) with the value of 0.39, 1.94, 3.57, 5.71, 8.00 and 10.26 cm against 0.0, 0.01, 0.31, 1.47, 3.17 and 5.35 cm at 5, 10, 15, 20, 25, and 30 days age of plant, starting after 12 days of sowing. The impact of the package bio-fertilizers have also able to decline late blight severity from 96.00 - 1.30 per cent in susceptible variety *Kufri sindhuri*. Biochemical analysis of plants have revealed that all the treatments significantly increased the soluble protein and total phenol content as compared to control at 5, 10 and 15 days after pathogen inoculation. Among the treatment, maximum amount of soluble protein and total phenol was found in case of T_7 (soil application of mustard cake + tuber treatment and foliar spray with *T. viride*) treatment, representing the value 30.38, 33.17, 32.41 and 2.10, 2.35 and 2.25 mg/g of fresh leave at 5, 10 and 15 days of pathogen inoculation. Correlation coefficient and regression equation revealed that negative correlation between disease severity and soluble protein and also between diseased severity and total phenol content as indicating (r) - 0.5224, -0.5842 and -0.5486 and -0.5360, -0.5656 and -0.3225 at 5, 10, 15 days of inoculation.

Keywords: Bio-fertilizers, potato, disease severity, biochemical changes, regression equation.

Potato is the world's fourth largest food crop, following maize, wheat and rice considered as "King of vegetables". The worldwide production of potato in 2014 is about 365365367 million tons (FAOSTATE 2012), whereas in India, total production is about 41555.4 million ton from 1973.2 million hectors of land with productivity of about 21.1 mt per hector which contribute 25.5 % of total area under vegetables. In India, Uttar Pradesh ranked first in potato production but as per concerned of productivity, the state is far behind than other country like Europe and America. The main reason of low productivity in India are diseases like early blight, late blight, wart, common scab, black scurf, black leg, virus, cyst nematode etc. Among them, late blight caused by *Phytophthora infestans* (Mont.) de Bary is the most destructive disease that had lead most infamous trophe as cat in Ireland (England) during 1840-45. The disease causes 22.6 - 80.90 % yield loss while losses in revenue varied from Kenyan Shillings 37,500 to 119,500 per hector but Losses upto 10 to 75 % have been reported in India. The pathogen is mainly perpetuated through tuber and soil through production of oospores. The management of the disease can be done through host resistance, cultural adjustments, biological and use of fungicides. But sometimes these

* To whom all correspondence should be addressed.
E-mail: samirkrbiswas@rediffmail.com

practices raise problem due to development of resistant strains of the pathogen which may become very difficult to control. Beside this, elevated temperature and CO_2 concentration due to climate change are also posing higher threat for management of several diseases of different crops (Gautam *et al.* 2013). Changing disease scenario due to climate change has highlighted the need of new strategies for sustainable food production. Development of new package and practices using bio-fertilizers are one of the main strategies for management of diseases in several crops (Ravindra *et al.*,2015, Yogesh Kumar, 2015, Biswas *et al.* 2008). The bio-fertilizers like *Azotobacter, Rhizobium,* PGR, *Trichoderma viride, Trichoderma harzianium,* Phosphorus solubilizing bacteria, fix the atmospheric N resulted also increase yields and growth in different crops (Singh *et al.*, 2001, Dhawan *et al*., 2005, Kachroo and Razdan 2006). Keeping in view the study was undertaken as "Development of suitable package using bio-fertilizers for management of late blight of potato under climate change"

MATERIALS AND METHODS

Seed treatment

Seed tubers were treated with *Azotobacter* @ 2g/10g of seed. 10gm Jaggery was also added to make slurry and mixed it with seed tuber. The tubers were then kept in shade for dry.

On the other hands, seed tubers were also treated with bio-formulation of *Trichoderma viride, Trichoderma harzianum* and phosphorus solubilizing bacteria separately. The seed tubers were treated by dipping the tuber in prepared solution @ 10 gm/ lit. of water separately. The treatments were given 2 hours before the sowing of tuber.

Effect of seed tuber treatment with bio-fertilizer on growth parameters and severity of late blight in potato

The experiment was conducted in the Glass house complex, Department of Plant Pathology, C.S.A. University of Agriculture and Technology, Kanpur. All these bio- fertilizers like *Azotobactor, T. viride, T. harzianum, phosphorus* solubilising bacteria were taken from Department of Soil Science & Agricultural Chemistry (Microbiology) and Department, of Plant Pathology, Chandra Shekhar Azad University of Agriculture and technology Kanpur. The seed tubers were collected from Vegetable Research Farm of this University.

The experiment was conducted using potato variety 'Kufri Sindhuri' and treatments were given separately as below:-

a) T1 = Soil application FYM @150gm/pot + neem Cake @150gm/pot + tuber treatment with *T. harzianum* + foliar spray with bio-formulation of *T. harzianum*
b) T2 = Soil application with FYM @150 gm/ pot + Tuber treatment with PSB + foliar spray with bio-formulation of PSB
c) T3 = Soil application FYM @150gm/pot + tuber treatment with Azotobacter + foliar spray with bio-formulation of Azotobacter
d) T4 = Soil application FYM @150gm/pot + mustard cake @ 150gm/pot + tuber treatment with Azotobacter + foliar spray with bio-formulation of Azotobacter
e) T5 = Soil application FYM @150gm/pot + neem cake @ 150gm/pot + tuber treat with PSB + foliar spray with bio-formulation of PSB
f) T6 = Soil application FYM @150gm/pot + tuber treatment with *T. harzianum* + foliar spray with bio-formulation of *T. harzianum*
g) T7 = Soil application FYM @150gm/pot + mustard cake @ 150gm/pot + tuber treatment with *T. viride* + foliar spray with bio-formulation of *T. viride*
h) T8 = Soil application with FYM @150gm/ pot + Tuber treatment with azotobacter + foliar spray with bio-formulation of Azotobacter
i) T9 = Soil application with FYM 300gm (Control).

The treated tubers were then sown in 30cm earthen pots, which were previously filled with a mixture of sterilized sandy loam soil and farm yard manure in the ratio of 2:1. In each pot one seed tuber was sown and watered as per need base. The experiment of design was laid out in simple CRD. Three replications per treatment and three pots were sown with untreated seed tubers served as control. The observations pertaining to the effect of different treatments on plant height at every 5 days upto 30 days age of plant and disease

severity (%) at 50, 55, and 60 days after sowing were taken.

Growth parameter

Plant height

Three plants were selected randomly from tagged plots. The shoot height was measured (in cm) from the soil surface at basal portion to tip of leaf with the help of meter scale a every 5 days upto 30 days age of plant. Three replications were kept for each treatment. The average of three plants height was divided by 3 for obtaining their mean to consider plant height.

Effect of bio-fertilizer on disease development

Inoculation of the crop

At 45 days age, plants were inoculated with spore suspension of *P. infestans*. The concentration of spore was maintained at 10^6 spore/ml. The spore suspension was prepared from seven days old culture of the pathogen. The homogenized, spore suspension were inoculated on the foliage of each plant. The inoculated plants were then kept on the bench of wire house. After two days of pathogen inoculation, plants were sprayed with solution of bio-fertilizer.

Spraying of Bio-fertilizers

In order to determine the efficacy of bio-fertilizers as seed treatment and foliar spray in controlling disease development, the plants were sprayed with solution of bio-fertilizers separately after 2 days of pathogen inoculation. For preparation of solution of bio-fertilizers, 10gm of each bio-fertilizer were taken and mixed in beaker along with 250 ml of distilled water. It was later filtered with muslin cloth and pure solution was prepared. At the time of spraying the solution were diluted in 750 ml of distilled water to make final solution of 1000 ml. Second spray of solution was given after 7 days of first spary. The observation pertaining to the effect of different treatments on disease severity (%) was taken at 50, 55 and 60 days after sowing.

Measurement of disease severity

Observation on severity of disease was taken after 5 days of pathogen inoculation. The disease severity was measure using 0-9 scale as described by Malcolimson, (1976). Ten leaves were randomly selected from the pot for measurement of disease severity. The leaves with 1-9% infection received 1, 10% infection received 2, 11-25% infection received 3, 26-40% infection received 4, 41-60% infection received 5, 61-70% infection received 6, 71-80% infection received 7, 81-90% infection received 8, 91-100% infection received 9

The disease severity of individual plants was calculated by following formula as describe by (Malcilimson, 1976).

$$\text{Disease severity PDI} \frac{\text{Sum of numerical rating}}{\text{Total number of leaves examined} \times \text{maximum rating}} \times 100$$

Biochemical changes in potato due to effect of bio-fertilizer and pathogenesis

The mature and fresh potato leaves were collected from different treatments and the changes in the content of soluble protein and total phenol in leaves were estimated at 5, 10, and 15 days after inoculation of the pathogen.

Estimation of soluble protein

The method developed by Lowry *et al.* (1951) was used with slight modification to determine the total soluble protein content.Potato leaves from different treatments were harvested, washed with distilled water several times and blotter dried before protein extraction. A quality of 1.0 gm of each sample cut into small pieces and grinded in pre-chilled pistil and mortar using 1:5 ratios of leaves and extraction buffer. The suspension was centrifuged at 10,000 rpm for 30 minutes at 4°C. The supernatant was collected. A quantity of 7.5 ml of the supernatant was transferred in a tube and mixed with 2.5 ml of sample buffer and used for protein estimation. The working standard solution was pipette out and 0.2, 0.6 and 1.0 ml of the solution were put into series of test tubes. A quantity of 0.2 ml, 0.6 ml and 1.0 of the sample extract was also pipette out and kept into other test tubes, separately. Then volume in all the tubes was made up to 1 ml with water. A tube with 1 ml of water served as a blank. Later on, 5 ml of solution C was added in each test tube and incubated at room temperature for 10 min. Thereafter, 0.5 ml of FCR was mixed well immediately and incubated at room temperature for 30 min in dark place. The absorbance at 660 nm against the blank was read and standard graph was drown to calculate the amount of soluble protein in sample and represented as mg/ g of fresh sample.

Estimation of total phenol

The accumulation of phenols in potato plants after treatment with different bio-fertilizers followed by inoculation of pathogen was estimated

following procedure developed by Bray and Thrope (1954). In this method the total phenol estimation was carried out with FCR, which was measured at 650 nm radiation calorimetrically.

For estimations, 1.0 gm of leaf sample of potato was ground in a pestle and mortar in 10 times volume of 80% ethanol. It was then centrifuged to homogenate the suspension at 10,000 rpm for 30 minutes at room temperature. Supernatant was separated and re-extracted for 5 times with required volume of 80% ethanol, centrifuged and the supernatant were pooled. It was then evaporated to dryness and residues were dissolved in 5 ml of distilled water. Different aliquots (0.2, 0.6 and 1.0 ml) were pipette out into test tubes and the volume in each tube was made to 3 ml with water. Subsequently 0.5 ml of FCR was added and after three minutes, 2 ml of 20% Na_2CO_3 solution in each tube was thoroughly mixed. Then absorbance at 650 nm against blank was measured using Ultra Violet Visible (UV-VIS) Spectrophotometer and the standard curve using different concentration of phenols was prepared. From the standard curve the concentration of phenols in the test sample was determined and expressed as mg phenols per gm of sample materials.

Correlation coefficient and Regression equation

The biochemical changes of potato leaves at different days of inoculation and disease severity of the corresponding days was analyzed to determine the level of association between soluble protein and disease severity as well as between total phenol and disease severity. The calculation was done by standard statistical calculation. Simple regression equations (Y= a + bx) were also developed for both the variables (protein and phenol) separately to understand their relation with disease severity.

RESULTS AND DISCUSSION

Plant Height

The effect of seed treatment with bio-fertilizers on germination and plant height of potato was studied and the data presented in table 2 showed that the seedling emerged out from soil at different days after sowing in different treatment. The early emergence and increase plant height of potato was found maximum in treatment T_7 (soil application of mustard cake + tuber treatment and foliar spray with *T. viride*) with the value of 0.40, 1.94, 3.57, 5.71, 8.00 and 10.26cm at 5, 10, 15, 20, 25 and 30 day age of plant starting after 12 days of sowing, followed by treatment T_4 (soil application of mustard cake + tuber treatment and foliar spray with *Azotobacter*) (0.09, 0.85, 2.41, 4.50, 7.64 and 10.18) and, T_1 (soil application of neem cake + tuber treatment with PSB) (0.52, 2.70, 4.14, 5.74, 7.93 and 9.89 cm). From the data presented in table 2 and 3, it is also cleared that all the treatments were able to increase the growth of plant over control. The result of experiments conducted by glass house shows that biofertilizer have a stimulatory effect on germination and vigor of plants. Kumar *et al.* (2001) found that investigated the establishment of strains of *A. Chroococum* including soil isolate and their mutants and reported that the strains of *A. Chroococum* better in all the varieties to increase

Table 1. Effect of Bio-fertilizers on germination and plant height of potato at different days after germination.

Treatment	Date of of germination	Plant height at different days after germination (cm)					
	After sowing	5 days	10 days	15 days	20 days	25 days	30 days
T_1	13 days	0.52	2.70	4.14	5.74	7.93	9.89
T_2	19 days	00	0.13	0.59	1.90	3.51	5.49
T_3	22 days	00	0.02	0.78	2.44	4.49	6.70
T_4	16 days	0.09	0.85	2.41	4.50	7.64	10.18
T_5	19 days	00	0.14	1.01	2.76	5.27	7.81
T_6	16 days	0.08	0.51	1.76	3.29	5.39	7.81
T_7	13 days	0.40	1.94	3.57	5.71	8.00	8.26
T_8	17 days	0.01	1.09	3.29	5.47	7.53	9.50
T_9	22 days	00	0.01	0.31	1.47	3.17	5.35
S.E.		0.26	1.11	1.44	1.67	2.31	2.51
C.D.		0.56	2.35	3.04	3.50	4.85	5.28

Table 1. Effect of bio-fertilizer on disease severity of late blight of potato

Treatment	Disease severity (%)		
	50 days	55 days	60 days
T1	5.23	9.48	12.51
T2	14.21	19.34	22.47
T3	9.32	13.31	16.69
T4	1.75	5.63	9.00
T5	9.00	13.05	16.18
T6	8.97	14.11	16.41
T7	1.30	4.21	7.82
T8	6.10	10.31	13.55
T9	62.03	83.84	96.00
S.E.	0.0912	1.4613	0.2136
C.D. (P=0.05)	0.1916	3.0701	0.4488

grain yield over control. Ravindra *et al.* (2015) found that the yield of tomato crop significantly increase by the combine application of seed treatment with *T. harzianum* + soil application of neem cake powder + foliar spray of Carbendazim.

Effect of tuber treatment and foliar spray with bio-fertilizer on severity of disease.

The effect of tuber treatment and foliar spray with bio-fertilizer on severity of disease revealed that there is a decline in late blight severity due to various treatments (Table- 1). The susceptible variety *Kufri sindhuri* of potato showed 96% disease severity, where as in case of treatment T_7 (soil application of FYM @ 150gm/pot+mustard cake @ 150gm/pot + tuber treatment and foliar spray with *T. viride*), the per severity

Table 2. Effect of bio-fertilizers on total soluble protein content in potato leaves after 5 days, 10days and 15 days of pathogen inoculation

Treatment	Total soluble Protein content (mg/g fresh leaves)			% increase over control
	5 days	10 days	15 days	
T1	30.07	32.28	31.39	46.27
T2	23.56	25.35	23.53	9.64
T3	24.21	26.41	24.49	14.11
T4	30.18	32.41	31.78	48.08
T5	25.7	27.56	26.41	23.06
T6	25.45	26.42	25.65	19.52
T7	30.38	33.17	32.41	51.02
T8	29.4	30.60	29.32	36.62
T9	20.75	22.48	21.46	
S.E.	0.1362	0.1624	0.1793	
C.D.	0.2863	0.3412	0.3767	

Table 3. Effect of bio-fertilizer on total phenol content in potato leaves after 5 days, 10days and 15 days of pathogen inoculation

Treatment	Total soluble Protein content (mg/g fresh leaves)			% increase over control
	5 days	10 days	15 days	
T1	1.70	1.91	1.92	27.15
T2	1.50	1.61	1.52	0.66
T3	1.53	1.71	1.63	7.94
T4	2.01	2.30	2.23	47.68
T5	1.55	1.74	1.66	9.93
T6	1.7	2.26	2.22	47.01
T7	2.10	2.35	2.25	49.00
T8	1.70	1.77	1.74	15.23
T9	1.49	1.59	1.51	
S.E.	0.1878	0.0261	0.0318	
C.D.	0.3947	0.0549	0.0670	

was only 1.30%. The treatment T_4 (Soil application FYM @150gm/pot + mustard cake @ 150gm/pot + tuber treatment with Azotobacter + foliar spray with bio-formulation of Azotobacter), showing 1.75 % disease severity representing second lowest among the treatment. The others treatment like T_1 (Soil application FYM @150gm/pot + neem cake @150gm/pot + tuber treatment with *T. harziaenum* $_+$ foliar spray with bio-formulation of *Trichoderma harzianum*) and T_8 (Soil application with FYM @150gm/pot + Tuber treatment with azotobacter + foliar spray with bio-formulation of Azotobacter) showing 5.23% and 6.10% disease severity, respectively at 50 days age of plant, which are also superior over control but inferior than T_7 and T_4 treatments. The decrease in disease severity might be the activity of biofertilizer which stimulate to synthesis of some defense compounds in potato against *P. infestans*. Similarity at 55 days and 60 days of plant the minimum disease severity was noted in $T_{7,}$ where treatments was given as soil application FYM @150gm/pot + mustard cake @ 150gm/pot + tuber treatment with *T. viride* + foliar spray with bio-formulation of *T. Viride*. Among the treatments maximum disease severity was noted in T_2 treatments followed by $T_{3,}$ representing 19.34, 22.47 and 13.31, 16.69% , respectively at 55 and 60 days age of plant. It is also cleared from the table that the disease severity continuously increases from 50 to 55 days and 55 to 60 days in all the treatments. Someya *et al*. (2006). Combined use of the biocontrol bacterium Pseudomonas fluorescens strain LRB3W1 with reduced fungicide application for the control of tomato Fusarium wilt. Surulirajan and Kandhaari,(2003) found that the effect of integrated treatment against rice sheath blight severity, disease incidence and yield parameter. Kumar and Dubey (2001) also developed management strategies of collar rot of pea by the integration of biological and chemical methods.

Biochemical changes associated with the effect of bio-fertilizer during pathogenesis at different days

Soluble proteins

Protein is an important defence molecules synthesised in plant during pathogenesis. The data presented in table 2 showed that the maximum amount of soluble protein contents was found in treatment T_7 (Soil application FYM @150gm/pot + mustard cake @ 150gm/pot + tuber treatment with *T. viride* + foliar spray with bio-formulation of *T. viride*) representing the value 30.38mg/gm , 33.17mg/gm and 32.41mg/gm of fresh leaves at 5, 10 and 15 days after pathogen inoculation against 20.75mg/gm, 22.48mg/gm and 21.46mg/gm at in case of control plant. Other treatments like T_4 (Soil application FYM @150gm/pot + mustard cake @ 150gm/pot + tuber treatment with Azotobacter + foliar spray with bio-formulation of Azotobacter (31.78mg/gm), T_1 (Soil application FYM @150gm/pot + neem Cake @150gm/pot + tuber treatment with *T. harzianum* $_+$ foliar spray with bio-formulation of *T. harzianum* (31.39mg/gm), T_8 (Soil application with FYM @150gm/pot + Tuber treatment with Azotobacter + foliar spray with bio-formulation of Azotobacter (29.32mg/gm) and T_5 (soil application FYM @150gm/pot + neem cake @ 150gm/pot + tuber treat with PSB + foliar spray with bio-formulation of PSB (26.41mg/gm) were also able to increased the soluble protein content by 48.08%, 46.27%, 36.62% and 23.06%, respectively over control. From the table, it is also cleared that all treatments increased protein content to a maximum at 10th day of pathogen inoculation, there

Table 4. Correlation of disease severity with total soluble protein and total phenol content of potato leaves

Biochemical Parameters	Days after pathogen inoculation	Correlation coefficient (r) with disease	Regression equation
Total soluble protein	5 days	-0.5224	Y= 31.2234
	10 days	-0.5842	Y=30.7855
	15 days	-0.5486	Y=30.6707
Total phenol	5 days	-0.5360	Y=2.1431
	10 days	-0.5656	Y=2.3013
	15 days	-0.3225	Y=2.2223

after it was decreased gradually from 10 to 15 days. The decrease protein content in diseased plants than healthy may be due to utilization of some protein by the pathogen. Antoniew *et al.* (1980) considered that pathogen related proteins (PR protein) are involved in plant defence response to pathogens. Boller (1985) was also opinion that proteins are are in the form of chitinase, PR-1 peroxides, β-glycosidase etc associated with defence response in plants against fungi and bacteria. Soluble protein is one of the most important defences compound synthesized with in plant as a response of biotic, abiotic inducers prior to infection by the pathogen (Kuc 1995, Biswas *et al.* 2012 and Razik *et al.* 2012). Singh and Prithviraj (1977) also found spraying of that neemazol, a product of neem before pathogen inoculation *Erysiphe pisi* increased protein content in pea leaves. Satesh Kagale *et al. (*2004) reported that neem leaf extract caused accumulation of polyphenol and PR-protein in rice crop.

Total phenol

Phenols are well known antifungal, antibacterial and antiviral compound. The phytoalexins are phenolic compound in their chemical constitution involved in plant defence. The resulted presented in table- 3 shows that all the treatment significantly increased the total phenol content as compared to control at 5, 10 and 15 days after pathogen inoculation. The maximum amount of phenol content was found in T_7 (Soil application FYM @150gm/pot + mustard cake @ 150gm/pot + tuber treatment with *T. viride* + foliar spray with bio-formulation of *T. viride*). against 1.49mg/g,1.59mg/g and 1.51mg/g in case of control at 5, 10 and 15 days, respectively. The percent increase in phenol content in T_6 treatment 49.00% higher than control plant (T_9) at 15th day of pathogen inoculation followed by treatment T_4 (Soil application FYM @150gm/pot + mustard cake @ 150gm/pot + tuber treatment with Azotobacter + foliar spray with bio-formulation of Azotobacter) and T_6 treatment (Soil application FYM @150gm/pot + tuber treatment with *T. harzianum* + foliar spray with bio-formulation of *T. harzianum*) with a value of 2.10mg/g, 2.35mg/g and 2.25mg/g of leaves Phenols are involved in plant defence in many ways like hypersensitive cell death or lignifications of cell walls were reported by several workers (Nicholson and Hamnerschidt, 1992; Kumawat *et al.* 2008; Arzoo *et al.* 2012). Matern and Kneusal (1988) suggested that the first stage of defense mechanism involve a rapid accumulation of phenol at the infection site which restricts or slows the growth of the pathogen.

Correlation coefficient and regression equation

The changes biomolecules in potato leaves under different growth stages and disease severity of the corresponding stage showed that reduced disease severity was associated with increased soluble protein and phenol content. The negative correlation (r) -0.5224, -0.5842 and -0.5486 was found between disease severity and soluble protein content at 5, 10, 15 days of pathogen inoculation. Similarly, correlation between diseased severity and total phenol content also showed negative correlation as indicating -0.5360, -0.5656 and -0.3225 at 5, 10, 15 days of inoculation. The corresponding simple regression equation also showed the negative relation between total protein and disease severity as well as total phenol and disease severity. The present findings are also supported by several workers. (Arzoo *et al*. 2012; Kumawat, *et al.* 2008, Biswas *et al*. 2012).

Thus the present study showed that use of bio-fertilizer has a good control ability of late blight of potato. The mechanism of defence response revealed that involvement of some defence molecules like the total proteins and phenols are synthesized due to application.of bio fertilizers which may be a better alternative of disease management in sustainable agriculture.

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