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Management of *Macrophomina phaseolina* (Tassi.) Goid. Causing Leaf Blight Disease in Mung Bean in Maharashtra State

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ABSTRACT

Leaf blight (LB) disease caused by *Macrophomina phaseolina*, an important constraint in mung bean production in India. In the present investigations, the aimed to evaluate the loss in yield, better mung bean cultivars against LB and the efficient management. Based on symptomatology, cultural and morphological characteristics the pathogen isolated from cv JL-781 it was identified as *M. phaseolina* (Tassi.) Goid. inducing LB disease. Pathogenicity of *M. phaseolina* was proved on the mung bean cv JL-781. Subsequently, field experiment was conducted for the management of LB disease using seed treatment with carbendazim 50 WP a.i. 0.2% (2 g/kg) + spraying of zinc sulphate monohydrate @ 0.01% + soil application of *Trichoderma viride* @ 5 g/ha mixed with 3 kg of FYM + foliar spray of garlic extract (10%) showed 15.45% incidence. This result was followed by 16.79% disease incidence and 15.44% disease severity by seed treatment with carbendazim, foliar spray of zinc sulphate, and soil application with *T. viride* @ 5 g/ha mixed with 3 kg of FYM.

Keywords Carbendazim, *Trichoderma* sp., Zinc sulphate, Mung bean, Leaf blight.

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INTRODUCTION

Mung bean (*Vigna radiata* L. Wilczek) is one of the most important pulse crops, primarily grown as staple pulse food in India. Pulses are rich in protein content (18 to 32%), which plays an important role in human and animal nutrition. Mung bean is extensively grown in Madhya Pradesh, Maharashtra, Uttar Pradesh, Andhra Pradesh and Tamil Nadu. The area, production and productivity of mung bean in India during 2018-19 was recorded about 4.2 M ha, 2.0 MT, and 472 kg/ha, respectively (Anonymous 2017). In Maharashtra, area, production and productivity of mung bean during the year 2018-19 was recorded about 3.97 lakh ha, 1.66 lakh tone and 361 kg/ha, respectively. Maharashtra shares 11% in total area

and 8% in the total mung bean production in India (Anonymous 2017).

Mung bean is known to infect by >35 fungal, a few viral, some bacterial and certain nematode species during seed germination to its maturity stages and resulting into substantial yield losses (Agarwal and Sharma 1989). Among the fungal pathogens, *M. phaseolina* is one of the major constraints in mung bean production in India (Khaire *et al.* 2021). Vidhyasekaran and Arjunan (1978) in India reported the LB disease occurrence in mung bean caused by *M. phaseolina* (Tassi.) Goid from Tamil Nadu. *M. phaseolina* is one of the important seed-borne pathogens inducing various symptoms including seedling blight, root rot, charcoal rot, wilt or dry root rot, stalk rot, stem blight, fruit rot, seedling decay or damping-off, crown rot and leaf blight on different crop plants. Kaushik and Chand (1987) estimated 2.2–15.7% infection in mung bean due to *M. phaseolina* leading to 10.8% and 12.3% loss of grain yield and protein content of the seed, respectively.

M. phaseolina infects >500 plant species including peanut (*Arachis hypogea*), beet (*Beta vulgaris*), cabbage (*Brassica oleracea*), pepper (*Capsicum annuum*), chickpea (*Cicer arietinum*), *Citrus* spp. *Corchorus* sp., *Cucumis* spp., *Fargaria* sp., soybean (*Glycine max*), *Gossypium* spp., sunflower (*Helianthus annuus*), sweet potato (*Ipomoea batatas*), alfalfa (*Medicago sativa*), *Phaseolus* spp., *Pinus* spp., *Prunus* spp., sesame (*Sesamum indicum*), potato (*Solanum tuberosum*), sorghum (*Sorghum bicolor*), bean (*Vigna unguiculata*), and maize (*Zea mays*) (Wyllie 1993).

Management of *M. phaseolina* is difficult due to long-term survival and persistence as a soil saprophyte and having wide host range. Therefore, management of *M. phaseolina* in different crops was achieved through integrated disease management approaches (Khaire *et al.* 2018). Among the various IDM approaches, preventive and curative measures using chemicals play an important role in the management of soil-dwelling and seed borne infections. In India and elsewhere therefore, mostly systemic and contact chemicals are routinely used for seed treatment and foliar applications in different crops (Khaire *et al.* 2018). Seed treatment of such chemicals including difenoconazole, carbendazim, carboxin, tebuconazole, and thiophanate methyl significantly effective against dry root rot caused due to *M. phaseolina* in different crops (Parmar *et al.* 2017, Khaire *et al*. 2018). Similarly, biocontrol agents like *Trichoderma* spp. and bacterial strains have been found effective against dry root rot, leaf blight, and charcoal rot diseases caused by similar pathogen in different crops (Deshmukh *et al.* 2016, Khalili *et al*. 2016, Torres *et al.* 2016). Recently, IDM approaches for management of *M. phaseolina* have been studied for different crops including chickpea (Dhingani and Solanky 2016, Lakhran *et al.* 2018), brinjal (Dapkekar *et al.* 2018), pigeonpea (Swamy *et al.* 2018), sesame (Ibrahim and Abel-Azeem 2015). Moreover, least information is available on sources of resistance, survey and surveillance, combined effect of chemicals, biocontrol agents, micronutrient and plant extracts against LB disease of mung bean in India. Therefore, by considering the impact of LB disease on yield in mung bean, IDM approaches were worked out in the present investigations.

MATERIALS AND METHODS

Collection of diseased leaf samples

During 2018-19 cropping season, surveys were conducted for recording the prevalence of LB disease in mung bean in 68 villages covering 272 farmer's field in Jalna and Aurangabad districts of Maharashtra, India. The disease incidence and severity were recorded from seedling to the crop harvesting stage. During survey, diseased leaves were collected from different locations and were stored at 4-6°C in the refrigerator for further investigation.

Isolation and pathogenicity

The isolation of the fungus was carried out by standard tissue isolation technique (Lakhran *et al.* 2018). The grown culture was stored at 4-6°C in refrigerator for further studies. Morphological and cultural characteristics of the fungus were based on the earlier reports on key characteristics (Prameela and Singh

Table 1. Effect of fungicide, micronutrient, botanical and bio-agent on leaf blight disease incidence and intensity in mung bean cv JL-781 during 2016-17 crop growing season. Note: Figures in parenthesis are arcsine transformed values, T_1-T_8: Treatments, ST: Seed treatment, FA: Foliar application, SA: Soil application, +: Plus.

Treatments	Method of application	Disease incidence (%) After Ist spray	Disease incidence (%) After 2nd spray	Disease incidence (%) Mean (%)	Disease intensity (%) After Ist spray	Disease intensity (%) After 2nd spray	Disease intensity (%) Mean (%)	Disease control (%) After Ist spray	Disease control (%) After 2nd spray	Reduction (%) over control	Yield* kg/ha
T_1: Carbendazim 2 g/kg	ST	26.79 (31.14)	25.10 (30.05)	25.94 (30.59)	25.05 (30.01)	21.07 (27.30)	23.06 (28.65)	33.14 (35.06)	45.96 (42.65)	39.55 (38.85)	1516
T_2: Zinc sulphate 0.01 %	FA	29.52 (32.89)	27.22 (31.42)	28.37 (32.15)	27.18 (31.40)	26.25 (30.79)	26.71 (31.09)	27.57 (31.61)	32.68 (34.75)	30.12 (33.18)	1404
T_3: *T. viride*	SA	27.09 (31.33)	25.22 (30.13)	26.15 (30.73)	25.27 (30.15)	22.99 (28.61)	24.13 (29.38)	32.40 (34.57)	41.52 (39.96)	36.96 (37.26)	1476
T_4: Garlic extract 10%	FA	28.28 (32.11)	27.57 (31.66)	27.92 (31.88)	25.51 (30.29)	24.55 (29.67)	25.03 (29.98)	28.97 (32.53)	37.00 (37.35)	32.98 (34.94)	1444
T_5: T_1 + T_2	ST + FA	23.61 (29.04)	23.03 (28.65)	23.32 (28.84)	22.14 (28.04)	19.88 (26.44)	21.01 (27.24)	41.09 (39.84)	49.00 (44.41)	45.04 (42.12)	1752
T_6: T_1+ T_2+ T_3 + T_4	ST + FA + SA + FA	16.48 (23.92)	14.51 (22.36)	15.45 (23.14)	15.06 (22.80)	12.72 (20.87)	13.89 (21.83)	59.71 (50.61)	67.47 (55.21)	63.59 (52.91)	2206
T_7: T_1+ T_2+ T_3	ST + FA + SA	17.45 (24.64)	16.13 (23.66)	16.79 (24.15)	16.90 (24.24)	13.98 (21.93)	15.44 (23.08)	55.05 (47.88)	64.12 (53.21)	59.58 (50.54)	2036
T_8: Untreated control	-	38.02 (38.04)	43.40 (41.19)	40.71 (39.61)	37.56 (37.78)	39.11 (38.69)	38.33 (38.23)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	1200
SEm±	-	00.73	00.68	-	00.81	00.79	-	01.62	01.73	-	0.24
CD @ 0.05	-	02.26	02.09	-	02.50	02.44	-	04.97	05.31	-	0.74

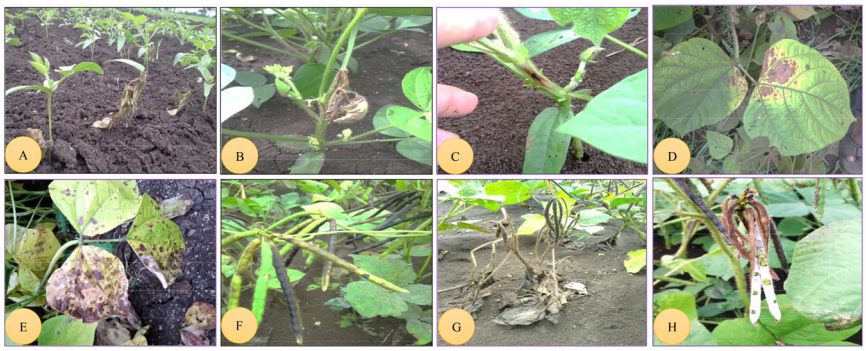
1998). In support of this microscopic observation at VNMKV, Parbhani, samples were also sent to Agharkar Research Institute (ARI), Pune. After the identification of the associated fungus *M. phaseolina* with the LB disease, the pathogenicity test was conducted under glasshouse conditions (Lakhran *et al.* 2018).

The spore-cum-mycelial suspension of *M. phaseolina* was prepared by harvesting 8 days old culture in 10 ml sterile distilled water and final concentration of 5×10^6 spores/ml was maintained. The 30 days old 2 seedling/pot were inoculated with *M. phaseolina* by uniform spraying by an atomizer and untreated control plants also maintained. All the inoculated and untreated seedlings were kept in glasshouse for eight days in which 80% relative humidity and 30±2°C temperature was maintained. Morphological and cultural characteristics of the fungus were examined by microscopic observations from the inoculated plants and the same pathogen was re-isolated as obtained from the field samples.

Management of LB disease in field condition

The field experiment was conducted in Randomized Block Design (RBD) at the experimental farm of the Agricultural Research Station, Badnapur, VNMKV, Parbhani during 2018-19 crop growing season using susceptible mung bean cv JL-781. A total of eight treatments were prepared with three replications each. Various combinations of biocontrol agent (*Trichoderma viride*), fungicide (carbendazim), garlic plant extract and zinc sulphate monohydrate as an essential micronutrient were used for management of the disease under field condition.

A total of eight treatments were prepared (Table 1). Two sprays of each treatment were undertaken at an interval of 15 days. One plot per replication was maintained as untreated control with a plot size of 5.5 m^2. Occurrence of LB disease was recorded before and after each spray of each treatment. Five plants per treatment per replication were used for recording the observations and from each plant three trifoliate



Figs. 1. Symptoms of leaf blight disease on mung bean cv JL-781 during 2018-19 crop growing season. A: Leaf blight appearance on leaves at seedling stage of the crop, B: Damping-off of the seedlings, C: Stem blight, D: Mild leaf blight symptom on the leaves, E: Severe leaf blight symptom of the leaves with yellowing, F: Blight appearing on the pods, G: Drying of the older blighted leaves and drooping and H: Shriveling of the pods and tip drying.

leaves from bottom, middle, top portion of main branch were selected for recording the observations. Blight disease severity (%) was calculated using 0-9 disease rating scale (Mayee and Datar 1986). After crop harvest, an observation on seed yield was recorded across all the treatments.

$$\text{Disease incidence (\%)} = \frac{\text{No. of plants showing disease symptoms}}{\text{Total number of plants observed}} \times 100$$

Based on numerical rating scale, disease severity was worked out by using formula given by McKinney (1923).

$$\text{Disease severity (\%)} = \frac{\text{Summation of numerical rating}}{\text{No. of leaves observed} \times \text{Maximum rating scale used}} \times 100$$

Where, numerical rating: Rating score × Number of samples in particular category.

Statistical analysis

Data from the present study was subjected to analysis of variance (ANOVA). The percent disease incidence data was arcsine transformed for analysis (Gomez and Gomez 1984). The standard error of mean (SEm±), standard error of deviation (SEd) and critical difference (CD) values were calculated by Randomized Block Design using the protocol described by Sheron *et al.* (1998).

RESULTS AND DISCUSSION

Symptomatology

LB affected mung bean plants were exhibiting various symptoms in different cultivars including blighting of the leaves (Fig. 1A), damping-off (Fig. 1B) and stem blight during the seedling stage of mung bean (Fig. 1C). Similarly, affected plants showed leaf blight appearance at the margin of the leaves (Fig. 1D) with small irregular brown spots on all over the leaf surface (Fig. 1E). Subsequently, the infected leaves drooped and dropped from the plants (Fig. 1F). At the various surveyed locations, intermittent rains followed by continuous dry spell resulted in the coalescence of the spots and which led to the large size lesions on all over the leaf surface and whole leaves showed blighted appearance (Fig. 1G). Contrastingly, with the decrease in temperature due to rains, reduced LB disease spread in some of the surveyed locations. Black pinheads of sclerotia were recorded on blighted portion of the leaves. The LB affected plants were characterized by shrivelling of the pods (Fig. 1H) which resulted into the reduced grain yield. Typical symptoms of LB disease of mung bean caused by *M. phaseolina* was characterized earlier by various workers in India and elsewhere (Grover and Sakhuja 1981, Tandel *et al.* 2015). Moreover, the morphological and cultural key characteristics for the identification

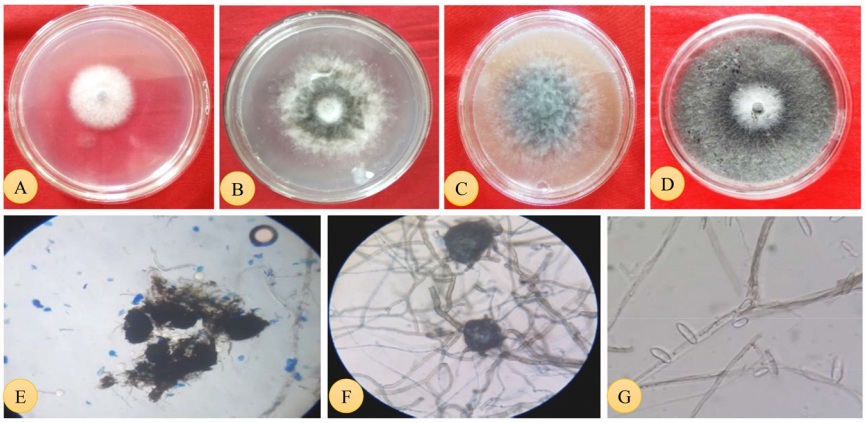


Fig. 2. Isolation and morphological identification of the *Macrophomina phaseolina* causing leaf blight disease in mung bean cultivars of Maharashtra, India. (A) At 3 days post inoculation (dpi), (B) At 5 dpi, (C) At 7 dpi (D) Micro sclerotial growth stage and microscopic observation of (E) Sclerotia, (F) Sclerotia with mycelial mat and (G) Macroconidia with apical appendages.

of the associated pathogen with leaf blight and root rot diseases in different crops were also described earlier (Thomber and Kohire 2018). These key characteristics were applied in the present investigations and observed the similar findings. Moreover, the pathogenicity of *M. phaseolina* was carried out in the present investigation was found similar with that of earlier findings (Thomber and Kohire 2018).

Isolation and identification of pathogen

Cultural characteristics of the isolated pathogen exhibiting the whitish and circular mycelial colony radiating towards the periphery which later turned brown in color (Fig. 2A-D). The sclerotia were observed at 8 days post inoculation (dpi). The sclerotia were dark brown to black in color (Fig. 2D). Shapes of sclerotia were ranged from spherical to oblong and to some extent irregular and size ranged from 120×95 µm (Fig. 2E). Based on the fungal morphological and cultural characteristics it was confirmed that the association of the *M. phaseolina* with the LB disease in mung bean in collected samples. Subsequently, to confirm the causal pathogen associated LB disease, the submitted culture samples at ARI, Pune and identified *M. phaseolina* as associated pathogen based on its cultural and morphological key characteristics. Moreover, microscopic observation of the fungal culture showed septate mycelium (Fig. 2F) which was initially observed as off-white in color later it turned into light brown to black colored mycelium and branched at the right angles with constriction at the base. Distance between two septa was ranged from 6 µm to 9.01 µm (Fig. 2G). The macro-conidia were seen having the apical appendages (Fig. 2G) as indicated with arrow. This confirmed the characteristics of the pathogen *M. phaseolina.*

Pathogenicity test

Pathogenicity test confirmed the Koch's postulate by inoculation of *M. phaseolina* on mung bean cv JL-781. The 15 dpi the inoculated plants were exhibiting the similar symptoms as like the symptoms recorded under field conditions (Figs. 3A-B). The re-isolated fungus from the inoculated mung bean cv JL-781 was identified as *M. phaseolina* based on morphological, cultural and microscopic key characteristics and thus, confirmed the association of *M. phaseolina* with the LB disease in mung bean in Maharashtra state, India.

Management of LB disease

Mung bean seed treated with T_6 treatment, T_1: Carbendazim 2 g/kg + T_2: Spray of zinc sulphate @ 0.01% + T_3: Soil application of *T. viride* + T_4: Spray of 10% garlic extract showed lowest disease incidence of about 15.45% as compared with the untreated control of about 40.71% (Table 1, Fig. 4). Similarly, second best treatment (T_7) was found effective that include T_1:

Figs. 3. Inoculation of *Macrophomina phaseolina* in mung bean cv JL-781 and recorded the symptoms as recorded from field condition (A) Foliar application of inoculum suspension and (B) Application of inoculum in the soil.

Seed treatment with carbendazim + T_2: spray of zinc sulphate @ 0.01% + T_3: Soil application with *T. viride* showed disease incidence of about 16.79%. This was followed by T_5, seed treatment with carbendazim as T_1 + T_2: Spray of zinc sulphate @ 0.01% showed LB disease incidence of about 23.32%. Nevertheless, application of garlic extract alone (27.92%) and zinc sulphate alone (28.37%) were found comparatively less effective than the combination with fungicides in reducing LB disease (Table 1, Fig. 4). After first spray of all the treatments, the LB disease incidence was ranged from 16.48% - 29.52% in the T_6 and T_2 as against 38.02% in untreated control (Table 1, Fig. 4).

Management of LB disease in different crops have been achieved through the application of *Trichoderma* spp. in chickpea (Dhingani and Solanky 2016, Khalili *et al.* 2016), bacterial strains (Torres *et al.* 2016), botanicals (Naik *et al.* 2020, Deshmukh *et al.* 2020) and different fungicides (Dhingani and Solanky 2016, Khaire *et al.* 2018). The application of fungicides, micronutrients, botanicals and biocontrol agents tested were found effective and significantly reduced disease severity as compared with the control. In the present study, the impact of different treatments was recorded after the first and second doses. Therefore, first dose of all the treatments seed including, seed treatment with carbendazim + spray of zinc sulphate @ 0.01%+ soil application with *T. viride* + spray of garlic extract @ 10% showed 16.48% incidence, which was the lowest incidence recorded in all the treated plants. Whereas, the maximum disease incidence of about 38.02% was recorded in the untreated control. This was followed by the best treatment including, seed treatment with carbendazim + spray of zinc sulphate @ 0.01% + soil application of *T. viride* showed 17.45% disease incidence.

Similarly, LB disease incidence was ranged from T_6: 14.51% to T_2: 27.22% after the second spray whereas, it was 43.40% in untreated control. Likewise, the seed treatment with carbendazim + spray of zinc sulphate @ 0.01% + soil application with *T. viride* + spray of garlic extract showed 14.51% incidence. Whereas, carbendazim + spray of zinc sulphate @ 0.01% + soil application with *T. viride* showed 16.13% incidence as compared with 43.40% in untreated control. This was followed by 23.03% - 27.57% incidence in rest of the treatments (Table 1). Least information is available in India similar to the treatments used in the present investigation and this has significantly reduced the incidence and severity of the LB disease in mung bean.

Disease severity and yield

In seed treatment with carbendazim + spraying with zinc sulphate + soil application with *T. viride* + spraying with garlic extract, the disease severity was found 15.06% as compared to 37.56% in the untreated control. This was followed by the 16.90% disease severity in carbendazim + spray of zinc sulphate @ 0.01% + soil application of *T. viride* showed whereas, 22.14% severity was obtained in T_5: Carbendazim + spraying with zinc sulphate showed (Table 1). After the second spray of all treatments, the LB disease severity ranged from 12.72% to 26.25%. The T_6 treatment showed the highest seed yield of 22.06 qtl/

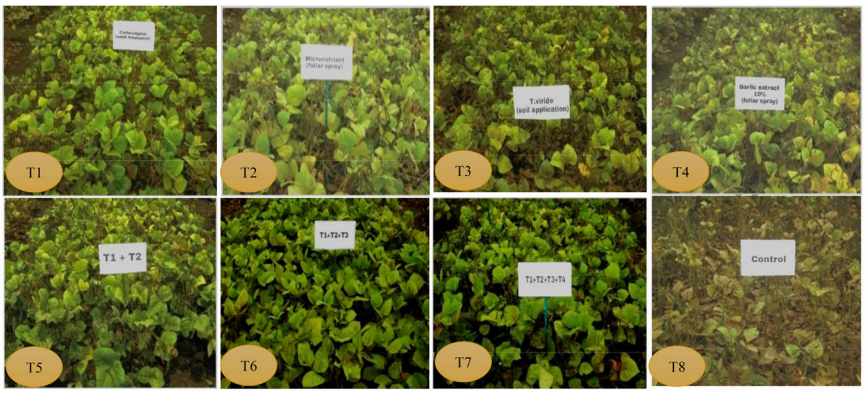


Fig. 4. Effect of fungicides, micronutrients, bio-agents and plant extracts on incidence and intensity of leaf blight disease on susceptible mung bean cv. JL-781.

ha as compared to 12.00 qtl/ha in untreated control (Table 1). Moreover, T_7 and T_5 showed at par yields of 20.36 qtl/ha and 17.52 qtl/ha, respectively (Table 1). Whereas, T_6 and T_2 treatments showed 12.72% and 26.25% severity, respectively as compared to untreated control (39.11%). This was followed by the 13.98% severity was recorded in the T_7 and subsequently, 19.88% with carbendazim + spray of zinc sulphate @ 0.01%, 21.07% with seed treatment with carbendazim alone. LB disease severity of 22.99% was recorded in soil application of *T. viride* alone, 24.55% by spraying with garlic extract and 26.25% by spraying with zinc sulphate alone. Dhingani and Solanky (2016) observed that the soil application of *T. harzianum* @ 5 kg in 500 kg neem cake/ha in furrow 5 days prior to sowing recorded 74.9% seed germination. While, soil application of *T. harzianum* @ 5 kg in 500 kg of FYM/ha in furrows five days before sowing of chickpea showed 11.81 disease incidence and yield 15.33 qtl/ha. Finding from the present study showed highest yield of 22.06 qtl/ha.

Therefore, from the present study it was inferred that the use of zinc sulphate, an enriched micronutrient, carbendazim a broad-spectrum fungicide, *T. viride* as a potential biocontrol agent and garlic plant extract as an efficient botanical have been found efficient against LB disease in mung bean in Maharashtra. The treatments have been significantly reduced the LB disease incidence and severity as compared to untreated control and thereby enhanced the total yield of mung bean.

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