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Effect of Soil Solarization, Chicken Litter and Viscera on Populations of Soilborne Fungal Pathogens and Pepper Growth

Sener Kurt and Betul Emir
Department of Plant Protection, Faculty of Agriculture,
Mustafa Kemal University, 31040, Hatay, Turkey

Abstract: The objective of this study was to determine the effects of soil amendments such as chicken litter and viscera in combination with soil solarization on populations of soilborne plant pathogens and pepper growth and yield. Solarization of plots supplemented with chicken litter and viscera was performed by mulching soil with a single layer of 0.030-mm-thick, clear, nonembossed, UV-stabilized, low density transparent polyethylene plastic sheets for 9 week in 2001. In the greenhouse conditions, the highest soil temperature were 54.8 and 53.6°C at Sites 1 and 2 in July, respectively. At Sites 1 and 2, chicken litter amendment to soil prior to solarization increased soil temperature by approximately 2-6°C, respectively, compared with the temperature of nonamended solarized soil at the 5 cm depth. There was little difference in soil temperatures between viscera amended and nonamended solarized plots at all depths of the soil. At both sites, pepper yield during 2001 to 2002 growing season in the amended solarized plots was significantly ($p=0.05$) higher than those in the nonsolarized plots. At a 5 cm depth, soil solarization had the highest effect on *F. oxysporum* and *Pythium* sp., with 78.6 and 56.7%, respectively, at Site 1. Solarization+viscera amendment was ineffective in suppressing *S. sclerotiorum*, *Pythium* spp. and *F. oxysporum*, except that *Phytophthora* spp. at all depths at Site 2. Soil solarization did not reduce population densities of *R. solani* and *Phytophthora* sp. at all depths. None of the soil treatments were effective in reducing population densities of soilborne pathogens at 25 cm depth.

Key words: Soil solarization, chicken litter, viscera, fungi, pepper

INTRODUCTION

Pepper (*Capsicum annuum* L.) is one of the most valuable horticultural crops produced in both the greenhouse and the field for fresh-market. Mature green peppers for spice consumption are also processed into powder in Turkey. Among the several yield-limiting pathogens in pepper production, *Phytophthora capsici* Leonian, *Sclerotinia sclerotiorum* (Lib.) de Bary, *Pythium* spp., *Fusarium oxysporum* Schlechtend. Fr., *Sclerotium rolfsii* Sacc., *Verticillium dahliae* Kleb. and *Rhizoctonia solani* Kühn are economically important soilborne plant pathogens with wide host ranges. Diseases caused by these pathogens, such as blight, white rot, damping-off, wilt, are a serious threat to vegetable crop production in the greenhouse in Turkey. Currently, these pathogens have frequently reached high inoculum densities, thus threatening crop production. A crucial factor in the management of fungal diseases caused by these pathogens is to reduce their inoculum level below critical threshold level before a susceptible crop is planted[1,2]. The main techniques used by growers to reduce losses due to these pathogens, especially at the early stages of plant development, are preplant soil fumigation with Methyl Bromide (MB) and postplant application of fungicides[3]. MB has been the most widely used chemical for the fumigation of soil, commodities, buildings and furniture for over 70 years[4]. These fumigants often lead to the eradication of beneficial organisms in soil and negative shifts such as health hazards, environmental pollution and potential atmospheric ozone depletion in the biological equilibrium. However, soils, especially those with a low microbial population, are more vulnerable to reinvasion of pathogens following fumigation. Increased environmental concern has been a major factor in triggering regulatory restrictions on the use of soil fumigants[5]. Under the Montreal Protocol, industrialized countries would phase out MB by 2005, with 25, 50 and 70% reductions in 1999, 2001 and 2003, respectively. However, developing countries would reduce production and use of MB by 20% in 2005 and totally phase out use by 2015[6]. Increased social and legislative pressure to restrict the use of MB, an effective soil fumigant used extensively to

Corresponding Author: Sener Kurt, Department of Plant Protection, Faculty of Agriculture, Mustafa Kemal University, 31040, Hatay, Turkey Fax: 00 90 326 2455832 E-mail: kurt@mku.edu.tr or senerk310450@yahoo.com

control a broad spectrum of pests, has created the impetus to evaluate alternative approaches for management of soilborne diseases[7-10].

Under conducive conditions and proper use, solarization can provide excellent control of soilborne pathogens in the field, greenhouse, nursery and homegarden. Soil solarization comprised of physical, chemical and biological components is an alternative approach to soil disinfestation which uses passive solar heating of moist soil mulched with plastic transparent polyethylene sheeting[11-13]. Soil solarization was first developed as a control for root disease in Israel and California[14,15]. Although soil solarization is used on a relatively smallscale worldwide as a substitute for synthetic chemical toxicants, its use is expected to increase as methyl bromide is phase out due to its ozone-depleting properties[12]. It results in increased health, growth, yield and quality of crop plants[16-18]. Although solarization has reduced populations of *Fusarium* spp., *Phytophthora* spp., *Pythium ultimum*, *Verticillium dahliae* and *Rhizoctonia* sp., it has not controlled other soilborne pathogens such as *F. oxysporum* f.sp. *lycopersici*, *Plasmodiophora brassicae*, *Pythium aphanidermatum*[8,19,20]. Soil solarization alone may not be consistently effective for the control of soilborne pathogens. In such cases, the combination of organic and inorganic soil amendments such as cruciferous residues, cow or poultry manure, chicken litter and other animal wastes have been used to enhance the performance of solarization[21-24]. Such compounds can stimulate germination of fungal propagules and increase microbial antagonistic activity in soil, thus encouraging biological control. Microbial activity against pathogens in soil can weaken propagules during solarization or suppress their re-establishment in soil and plant roots after treatment[6]. A common form of poultry manure is chicken litter, which consists of manure and pine shaving bedding. Chicken litter contains significant quantities of N, P, K, Ca, Mg and micronutrients and can be used as a substitute for commercial fertilizers[24,25]. Fresh chicken manure was highly effective in reducing the incidence of potato scab, Verticillium wilt and populations of parasitic nematodes[26]. However, little information is available on the effects of chicken litter and viscera in combination with solarization on control of certain soilborne plant pathogenic fungi in pepper growing based on environmentally friendly alternatives to replace methyl bromide in the greenhouse. The objective of this study was to determine the effects of soil amendments such as chicken litter and viscera in combination with soil solarization on soilborne plant pathogen populations and pepper growth and yield.

MATERIALS AND METHODS

Solarization sites: In 2001, greenhouse experiments were performed in commercial pepper production areas located in Samandag County, Hatay where soilborne plant pathogenic fungi had been seriously problem during 1999-2000. The experimental plot on Site 1 was previously cropped with pepper and was not known to infest with major soilborne plant pathogens other than *Phytophthora capsici*. The soil is loamy fine sand with low organic matter and pH value of 8.2. The experimental plot arranged on Site 2 has soil with a history a severe epidemic of white rot of pepper, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. Soil type on Site 2 was sandy clay with near 9.0 pH.

Experimental design and soil solarization treatments: Prior to treatments, greenhouse soils were deep-plowed and cultivated. Drip irrigation lines were installed before any transparent polyethylene plastic sheets were mulched. These plots were irrigated and maintained wet by repeated irrigations at 3 to 6 day intervals until mulching was removed. Plots were arranged in a Randomized Block Design with four replicates per treatment. Chicken litter (chicken excrement and pine shaving bedding) and viscera were obtained from commercial sources such as chicken production farms and sell shops in Samandag and Antakya Counties. The plots were preirrigated with 5 cm of water 8-10 days prior to covering the soil with polyethylene mulches. Chicken litter (at a rate of 10 t ha^{-1}) and viscera (at a rate of 0.5 t ha^{-1}) were normally spread on the plots, later incorporated into the soil by plowing. Solarization of plots supplemented with chicken litter and viscera was done by mulching soil with a single layer of 0.030-mm-thick, clear, nonembossed, UV-stabilized, low density transparent polyethylene plastic sheets. Solarization was performed for a period of 9 week, starting 12 July 2001. Daily temperature fluctuations in both solarized and untreated (nontarped) soils were continuously monitored at depths of 5, 15 and 20 cm in the center of each plot using a digital thermometer with stainless steel sensor probe (LCD Digital MULTI-STEM Thermometer, Germany).

Polyethylene sheets were removed from solarization treatments on 17 September 2001 after approximately 65 days. Each plot consisted of six east-west-oriented rows, 5 m long, with 1.0 m between rows, 0.2 m between plants in a row. The pepper crop Cv. Geyik boynuzu was planted in soil prepared according to standard commercial production practices in mid-October 2001 and drip irrigation lines were installed in the greenhouse. All plots were fertilized according to soil test recommendations.

Evaluations of plant growth, yield and disease incidence: Yield values were collected from the middle two rows of each plot. For each treatment, the total number of marketable fruits was counted and their weight was determined. In addition, plant development was measured in each plot. For fungal isolation, root and stem tissues of affected plants were washed under running tap water. Plant pieces taken from the lower hypocotyls and upper taproot were surface-sterilized in 1% NaOCL solution for 1 to 2 min, rinsed twice in sterilled distilled water and dried between sterilled filter papers. Pieces of disinfested tissues were plated on PDA amended with 200 μg mL^{-1} streptomycin sulfate, 10 μg mL^{-1} rifampisin and 100 μg mL^{-1} amoksisilin. The plates were incubated at 25°C for 7-10 days.

Quantification of microbial population density in soil: The soil was randomly collected from the 5, 15 and 25 cm of 8-10 sites in each experimental plot (5 kg per plot), placed in nylon bags by depth for each plot, combined, thoroughly mixed, air-dried for 48 h and passed through a 2 mm mesh screen, then stored in the shade at room temperature until it was analyzed.

Inoculum densities of the soilborne pathogens and the effectiveness of treatments were determined by assaying populations of fungi just before planting of pepper seedlings to the experimental plots. The populations were estimated in four 9 cm diameter petri dishes containing the appropriate medium by the soil dilution method (up to 10^{-3} dilution) for microbial enumeration. All of the population densities were compared with assays of nonamended plot soil served as controls at the same time. Potato Dextrose Agar (PDA) (potato, 250 g; dextrose, 20 g; agar, 15 g and distilled water, 1000 mL; pH 7.0), czapek-dox agar ($NaNO_3$, 2 g; K_2HPO_4, 1 g; $MgSO_4.7H_2O$, 0.5 g; KCl, 0.5 g; $FeSO_4$, 0.01 g; sucrose, 30 g; agar, 20 g; distilled water, 1000 mL) and corn meal agar (corn meal, 30 g; agar, 20 g; distilled water, 1000 mL) were used for this study. Ten gram of the soil sample were vigorously shaken in a sterile Erlenmeyer flask with 90 mL of distilled water on a reciprocal shaker for 1 h. Serial dilutions (1:10, 1:100, 1:1000, w/v) of the mixture were made in sterile distilled water and analyzed. From each dilution, a 1 mL soil subsample were transferred and spread onto each of five petri plates with a flammed L-shaped rod. Colonies of the fungi were counted under a dissecting microscope after incubation for 7 to 10 days at 24±2°C in the dark. The observed inoculum level is expressed as the number of Colony Forming Units (CFU) per unit of weight of dry soil. At least two sets of analysis were performed for each amendment and soil solarization combinations tested. The effectiveness (%) of treatments was calculated by assaying populations of different pathogens in the soil. All data were analyzed by using statistical software (SPSS, Inc., LEAD technologies, Chicago, IL) for personal computers with analysis of variance (ANOVA) to compare experiments and also to test the effects of amendments and soil solarization and means were separated by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Soil temperature: During the 9 week of soil solarization treatments, the main soil temperature fluctuation in solarized and nonsolarized (nontarped) plots is summarized in Fig. 1 and 2.

Based on the average maximum soil temperatures recorded during the solarization period in the greenhouse conditions, the highest soil temperature were 54.8 and 53.6°C at Sites 1 and 2 in July, 2001, respectively. However, at Site 1 and 2, chicken litter amendment to soil prior to solarization increased soil temperature by approximately 2 to 6°C, respectively, compared with the temperature of non-amended solarized soil at the 5 cm depth. In a previous study[2], the temperature of solarized soil amended with compost was reported to be very effective in increasing by 2-3°C, compared with the temperature rise in solarized, non-amended soil. At Site 1, the mean temperatures at depths of 15 and 25 cm in the chicken litter amended solarized soil were 49.5 and 43.6°C, compared with 42.8 and 37.5°C, respectively in the nonamended solarized soil. At Site 2, the mean temperatures at depths of 15 and 25 cm in the chicken litter amended solarized soil were 45.0 and 38.1°C, compared with 38.4 and 34.4°C, respectively, in the nonamended solarized soil. There was little difference in soil temperatures between viscera amended and non-amended solarized plots at all depths of the soil. At the 25 cm depth, the soil temperature was similar in the nonamended solarized, chicken litter amended and viscera amended solarized plots except that nonamended nonsolarized plot. However, the mean soil temperatures at all depths were greater in plots that were solarized than in plots that were not solarized at both sites. These results are consistent with previous reports concerning that maximum soil temperatures exceeded 50°C at depths of 5 cm in experiments conducted in California, Arizona and North Carolina[7,19,27]. In addition, at a depth of 15 cm, the maximum soil temperature of 49.9°C recorded in this study was within the 44-52°C maximum temperatures reported from California[28, 29] and Israel[14]. Apart from irrigation intensity, air temperature and plastic film color, other factors play roles in determining the extent of soil heating via solarization. These include soil moisture and humidity

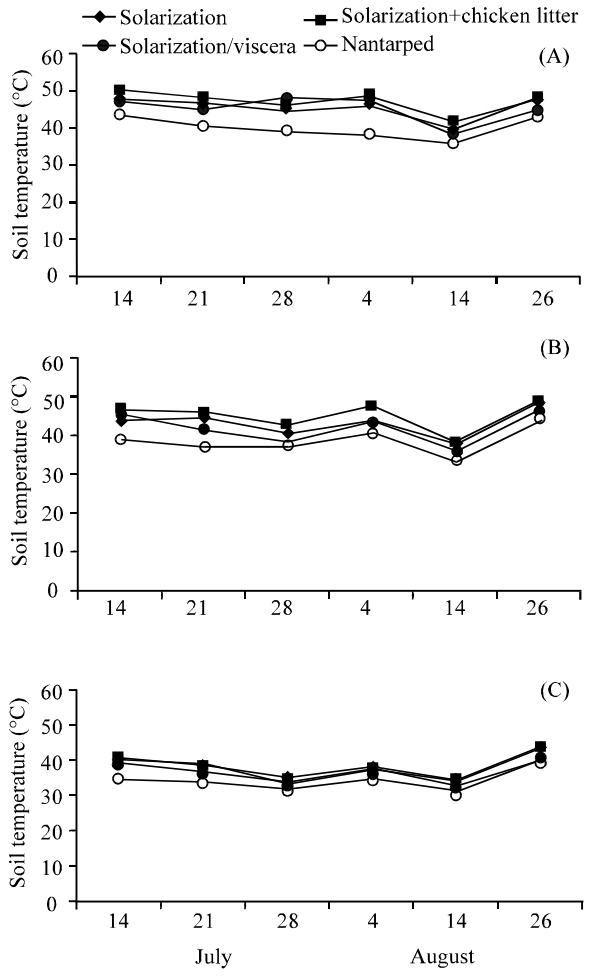

Fig. 1: Average daily minimum and maximum temperatures recorded during the 9 week solarization period at the depths of 5 (A), 15 (B) and 25 cm (C) in solarized treatment and untreated soil (nontarped) plots in 2001 at the Site 1. These plots were either covered with a single layer of the 0.030-mm-thick, clear, UV-stabilized, low densities polyethylene plastic sheets for 9 week during the growing season or left nonmulched

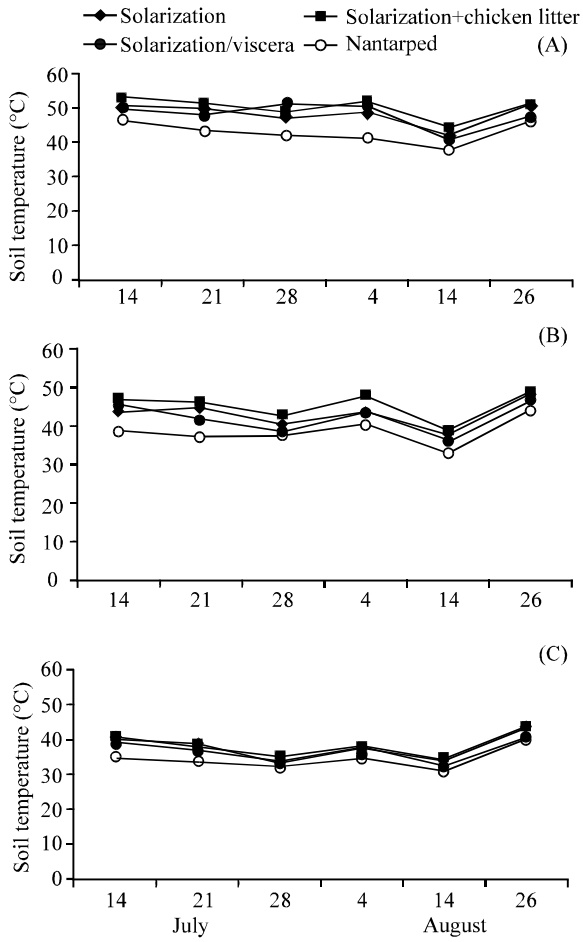

Fig. 2: Average daily minimum and maximum temperatures recorded during the 9 week solarization period at the depths of 5 (A), 15 (B) and 25 cm (C) in solarized treatment and untreated soil (nontarped) plots in 2001 at the Site 2. These plots were either covered with a single layer of the 0.030-mm-thick, clear, UV-stabilized, low densities polyethylene plastic sheets for 9 week during the growing season or left nonmulched

at the soil/trap interface, properties of the plastic, soil properties, color and tilth and wind conditions[12].

Plant growth and yield: Plant growth increases in plots planted with pepper immediately after solarization were significantly (p=0.05) higher than those in the nonsolarized plots at each site (Table 1). There were no significant differences in the plant development among soil amendment treatments. Fruit yield of pepper during 1999 to 2000 growing season in the amended solarized plots was significantly (p=0.05) higher than those in the nonsolarized plots. Differences in fruit yield could not be detected among the soil solarization alone, or solarization with chicken litter and viscera.

In agreement with the results of these studies, previous researches revealed that soil solarization frequently results in improved plant growth and yield increase[29-31] and in induced suppressiveness against reestablishment of major and minor fungal plant pathogens in amended soil[16,32,33]. In addition, cotton

growth and lint yield were improved with application of poutry litter at all locations[34] and increased as chicken litter rates increased regardless of when the litter was incorporated[24].

Quantification of microbial population densities in soil: The results of soil analysis for soilborne pathogens recovered from soil placed in nylon bags revealed that soil amendments with soil solarization had a significant effect on survival and population densities of pathogens. At a 5 cm depth at Site 1 (Table 1), soil solarization had the highest effect on *F. oxysporum* and *Pythium* sp., with 78.6 and 56.7%, respectively. In addition, solarization+chicken litter had an effect on same pathogens with 67.9 and 63.3%, respectively. Solarization+chicken litter amendment was the only treatment that was consistently effective in suppressing *Pythium* sp. with 55.2% at a 15 cm depth. Soil solarization did not reduce population densities of *R. solani* and *Phytophthora* sp. at all depths. Populations of these pathogens were significantly reduced in solarization amended with chicken litter plots. Solarization amended viscera had a significantly effect on *Pythium* sp., *F. oxysporum* and *Phytophthora* sp. at a 5 cm depth. At a 5 cm depth of Site 2 (Table 2), While solarization+chicken litter had the highest effect on *S. sclerotiorum* and *Phytophthora* spp. at 5 and 15 cm depths, solarization and solarization+chicken litter had the highest effect on *Pythium* spp. and *F. oxysporum* with 52.1-62.7 and 62.8-59.7%, respectively. Solarization+viscera amendment was the only treatment that was ineffective in suppressing *S. sclerotiorum*, *Pythium* spp. and *F. oxysporum*, except that *Phytophthora* spp. at all depths. None of the soil treatments at both sites were effective in reducing population densities of soilborne pathogens at 25 cm depth (Table 2 and 3).

The thermal decline of population densities of soilborne pathogens during solarization depends upon both the soil temperature and the exposure time[35]. Results obtained from this experiment are consistent with previous report[14] concerning that soil solarization reduced populations of *F. oxysporum* f.sp. *lycopersici* by 94-100% at a 5 cm depth, 67-100% at a 15 cm depth and 54-74% at a 25 cm depth. However, previous studies demonstrated that *Phytophthora* spp. were the most sensitive organism to temperatures achieved by soil solarization[8,29,36,37]. In another studies, solarization of soil amended with chicken manure was widely reported to be very effective in controlling similar to that achieved with methyl bromide of several soilborne diseases such as *Fusarium* wilt of Sweet basil[2,5,21,22].

In conclusion, these results are especially encouraging for future pepper production in Southern Turkey where soilborne diseases are a major problem. Although increased efficacy of soil solarization by chicken litter and viscera amendments on the reductions of populations of soilborne fungal pathogens was

Table 1: Effects of soil solarization, chicken litter and viscera on pepper growth and yield (*Capsicum annuum* Cv. Geyik Boynuzu) at each site after the solarization period in 2001-2002

	Site 1			Site 2		
Soil treatments	Plant height (cm)[a]	Branches (No./plant)	Fruit yield (kg m^{-2})	Plant height (cm)	Branches (No./plant)	Fruit yield (kg m^{-2})
Soil solarization	28.0a	6.9b	8.5a	29.3a	7.8a	8.9a
Solarization+chicken litter	26.7a	7.4a	8.8a	31.6a	8.3a	9.4a
Solarization+viscera	29.0a	7.5a	8.9a	32.8a	8.0a	9.3a
Nonsolarized	24.1b	8.0a	7.7b	23.2b	7.3a	7.4b

[a]Treatment means followed by the same letter are not significantly different according to Duncan' s Multiple Range Test at p=0.05

Table 2: The effect (%) of soil applied treatments on population densities of soilborne plant pathogens in pepper production at Site 1

Soil treatments	Soil depths (cm)	*Rhizoctonia solani*	*Pythium* spp.	*Fusarium ixysporum*	*Phytophthora* spp.
Solarization	5	12.3b*	56.7a	78.6a	11.0b
	15	43.2a	17.9b	26.2b	29.7ab
	25	0.0c	0.0c	0.0c	0.0c
	Mean	18.5	24.9	34.9	13.6
Solarization+	5	69.3a	63.3a	67.9a	70.3a
Chicken litter	15	28.0ab	55.2a	18.0c	45.3b
	25	13.9b	0.0b	0.0c	0.0c
	Mean	37.1	39.5	28.6	38.5
Solarization+	5	21.1a	60.0a	44.3a	54.2a
viscera	15	1.5b	13.4b	42.6a	14.1b
	25	1.6b	3.2c	38.5ab	9.4bc
	Mean	8.1	25.5	41.8	25.9

*Treatment means were seperated using Duncan's Multiple Range Test (p=0.05). Differences among treatments with the same letter(s) within a column could not be detected

Table 3: The effect (%) of soil applied treatments on population densities of soilborne plant pathogens in pepper production at Site 2

Soil treatments	Soil depths (cm)	*S. sclerotiorum*	*Pythium* spp.	*Fusarium oxysporum*	*Phytophthora* spp.
Solarization	5	16.9a*	52.1a	62.7c	6.2a
	15	25.0a	37.9b	52.0c	8.2a
	25	0.0 b	0.0c	0.0d	0.0b
	Mean	27.2	39.9	54.6	8.9
Solarization+	5	69.9b	62.8b	59.7a	71.6a
Chicken litter	15	43.8c	51.6b	32.0b	55.3b
	25	49.6c	0.0c	0.0c	0.0c
	Mean	53.4	54.8	36.2	60.1
Solarization+	5	19.3a	12.8a	31.3 b	54.3a
Viscera	15	26.0b	13.7a	33.3b	50.6a
	25	37.4b	8.5b	32.9b	52.2a
	Mean	28.6	11.7	32.6	52.3

*Treatment means were seperated using Duncan's Multiple Range Test (p=0.05). Differences among treatments with the same letter within a column could not be detected

observed, these reductions were not enough to eliminate pathogens at the same depths as methyl bromide. Therefore, other alternatives such as chemical and non-chemical approaches must be urgently searched to enhance the efficacy of soil solarization for disease control to replace the use of soil fumigants in agriculture.

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