

Asian Journal of Food and Agro-Industry

ISSN 1906-3040

Available online at www.ajofai.info

Enhancement of soil microbial metabolic activity in tomato field plots by chitin application

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Abstract

The influence of chitin application on soil microbial metabolic activity was investigated in tomato field plot experiments. Periodical observations were made in tomato planting soil beds amended with either 500g chitin or 1,000g chitin per 1 x 6m plot as well as in unamended soil bed control. Results obtained by the measurement of enzymatic activity such as dehydrogenase and chitinase in the tomato rhizosphere were compared and showed that the highest level of both enzyme activities were in the 1,000g chitin amended plots at 4 weeks after tomato transplanting. Determination of total microbial activity by fluorescein diacetate hydrolysis was also conducted to examine organic matter turnover. A similar increasing pattern was gained with 1.12, 0.73 and 0.22 times from 1,000g chitin, 500g chitin amended and unamended plots, respectively. The higher rates of enzyme activities and total microbial activity were observed in chitin amended soils even after harvesting period. Hence the application of chitin is markedly responsible for sustainable soil microbial activities enhancement.

Keywords: organic okra; conventional okra; vacuum fry; moisture heating; organic quality

Introduction

The use of organic amendments have been accepted to be the best for increasing soil fertility and nutrient supplies. Increasing of desirable properties such as soil organic matter

content, biomass C, a ratio of biomass C to total organic C, pore size distribution, aggregate cohesion, and the soil microbial activity have been reported (Pascual et al., 1997, Marinari et al., 2000, Lee et al., 2004). These characteristics are undoubtedly responsible for supporting crop growth and yields. Chitin is a naturally occurring aminopolysaccharide that can be commonly found as a major component of invertebrate exoskeletons and of fungal hyphae. This organic compound is known to be the most abundant renewable resource on the earth after cellulose. A main source for general production of chitin is derived from fisheries industrial waste containing a large amount of squid pens and shells of crab and shrimp. The chitin biopolymer exhibits a unique combination of low toxic physico-chemical and biological characteristics. The polysaccharide nature of chitin determines their biocompatibility and the reactive functional groups endow chitin with certain features that have important practical applications. Chitin is insoluble in common solvents but its decomposition is acquired by biological reaction. Degradation of chitin in soil is essentially a microbial process involving several hydrolytic enzymes and a variety of beneficial soil microorganisms including fungi, actinomycetes, and unicellular bacteria that are able to hydrolyse chitin (Gooday, 1990, Cody et al., 1990, De Boer et al., 1999).

Amendment of soil with chitin was first introduced for biological control of plant pathogenic fungi because of its capability in the stimulation of mycolytic microorganisms growth and replication. Reduction of fungal pathogens was assumed to be an effect of chitinolytic microorganisms that were subsequently increased in response to the addition of chitin. In addition, other organic acids, minerals and volatile compounds could also be released during a biodegradation process, for instance, acetate, propionate, peptides, nitrate and NH_3 that may contribute to pathogenic fungal suppression (Mitchell and Alexander, 1962, Schippers and Palm, 1973). Changes in bacterial communities and control of plant parasitic nematodes as well as soil borne plant pathogenic bacteria had been proved to be mediated by chitin application (Hallman et al., 1999, Sarathchandra et al., Homkratoke and Wongkaew, 2006). These studies on chitin amendment were mostly emphasized on the occurrence and population of available chitinase producing microorganisms by the assumption that the responsible chitinase formation in soil is directed by degradation of the chitin applied. The effects on chitinase activity in soil following chitin amendments were revealed using buried litter bags containing chitin and chitin amended compost (Krsek and Wellington, 2001, Poulsen et al., 2007). The chitinase activity, however, was found differ among soil samples taken for chitin amendment in spite of their identical textures and the addition of chitin appeared to stimulate both the chitinolytic and non- chitinolytic microorganisms that may compete for chitin oligomer released (De Boer et al., 1999). Microbial activity besides chitinase may also associate for chitin decomposition corresponds to overall nutrient cycling and plant protection effects. A dehydrogenase enzyme assay has been widely accepted to measure microbial activity in soil and sediments because the assay represents a broad group of enzymes that transfer hydrogen and electrons from substrates to appropriate acceptors. The method has been effectively used as an indicator of the primary activity of microorganisms during a biodegradation (Rossel et al., 1997, Mathew and Obbard, 2001) Another well known assay for measurement of total microbial activity in arrange of environmental sample including soils is fluorescein diacetate (FDA) hydrolysis. The enzymes responsible for FDA hydrolysis are plentiful in soil environment including non-specific esterases, proteases and lipases that are involved in decomposition process. Measurement of these decomposer activity, therefore, provide an accurate estimation of total microbial activity as more than 90% of the energy flow in soil system passes through microbial decomposers (Adam and Duncan, 2001).

The objectives of the present study were to investigate the influence of a biomaterial chitin amendment in sandy soil tomato field plots through-out the growing season. The assays on microbial metabolic activities including dehydrogenase, chitinase and the total microbial activity were used as indicators for substantial effects.

Materials and Methods

The effect of chitin amendment application on soil microbial metabolic activity was investigated in tomato field plot experiments on a sandy soil of the Yasothorn series. Raised soil bed plots 1 m wide, 6 m long and 0.3 m high were constructed. The soil beds were amended with chitin at rates of 1,000 g / plot or 500 g / plot one week before tomato transplanting. The treatments were replicated three times in a complete randomized block design, with control plots without chitin amendment.

Soil sampling

At least twenty sub-samples of 2.5 diameter x 10 cm deep soil cores were taken from each experimental tomato field plot. The soil sub-samples were then combined into one composite sample. The bulked samples were sieved (4 mm) and mixed before storage at 4°C until further manipulation.

Dehydrogenase activity

The dehydrogenase activity of each soil sample taken from either chitin amended plots or unamended plots was measured by colorimetric method using 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazoliumchloride (INT) as a substrate (Mathew and Obbard, 2001; Neto et al., 2007). Each 2 g of dry soil sample was sieved at 2 mm, with 1 ml of 2mM INT and 2 ml of Tris-HCl buffer pH 8 and was incubated away from the light in a waterbath at 37°C for 24 hours. The mixture was centrifuged at 1,735 g for 10 minutes and the collected pellets were extracted for formazan yielded by adding 20 ml of methanol. The extracted was further centrifuged at similar condition and the resulting supernatant was filtered using Whatman 112 V filter papers. The produced formazan was then assayed by measuring its absorbance at 493 nm and the result was expressed as microgram of INTF per gram of soil sample.

Chitinase activity

Chitinase activity of the soil samples was determined according to Badiane et al.(2001) that a *para*-nitrophenyl *N*-acetyl glucosaminide was used as substrate for colorimetric measurement. A sample of 100 mg each, was incubated for 2 h at 37°C, with 100 µl of 5 mM *para*-nitrophenyl *N*-acetyl glucosaminide and 400 µl of a citrate phosphate buffer at pH 5.8. The reaction was stopped with 3 ml of 0.2% Na₂CO₃ (w/v). The *para*-nitrophenol (PNP) released by chitinase activity was measured at 400 nm. Results were expressed as µg PNP released g⁻¹ h⁻¹.

Flourescein diacetate (FDA) hydrolysis

To determine the total microbial activity in soil samples, a sensitive and rapid method of fluorescein diacetate (FDA) hydrolysis described by Adam and Duncan (2001) was used. Two grams of less than 2 mm sieved soil sample was placed in a 50 ml conical flask and 15 ml of 60 mM potassium phosphate buffer pH 7.6 was added. A stock solution of 0.2 ml 1,000 µg FDA/ml was added to start the reaction. The mixture was then incubated at 30°C for 20 minutes in an orbital incubator at 100 rev/min. Once removed from the incubator, 15 ml of chloroform/methanol (2:1 v/v) was added immediately to terminate the reaction. The mixture was shaken thoroughly and subjected to centrifugation at 2,000 rev/min for 3

minutes. The supernatant obtained was filtered and the filtrate was measured for fluorescein released during the assay per gram of soil sample by its absorbance at 490 nm.

Statistical analysis

Data was subjected to an analysis of variance. The mean values were grouped for comparisons and the least significant differences among them were calculated at $P < 0.05$ confidence level using SPSS statistics 17 (2009 SPSS Inc., Chicago, Illinois 60606, USA.)

Results

Dehydrogenase activity

Dehydrogenase activity of the soil gradually increased during the growth of the tomato plants, but there was significantly different values and rates among the experimental plots. As shown in Figure 1, the soil dehydrogenase activity ($\mu\text{g INTF/g soil/24 hrs}$) in control plot were slightly increased with 0.32 and 0.21 times higher at 4 weeks after transplanting and one week after harvesting than the value measured one week before transplanting. The highest increasing rates were observed in the 1,000g chitin amended tomato soil plots with 3.46 and 0.64 times higher, follow by the case of 500g chitin amended tomato soil plots which were 2.64 and 0.63 times higher at the corresponding values and growing periods, respectively. Measurement of the dehydrogenase activity at the last period was also observed at one week after harvesting and their levels were 0.635, 0.628 and 0.21 times in the case of 1,00g chitin, 500g chitin amended plots and unamended plots, respectively, higher than the level at one week before tomato transplanting.

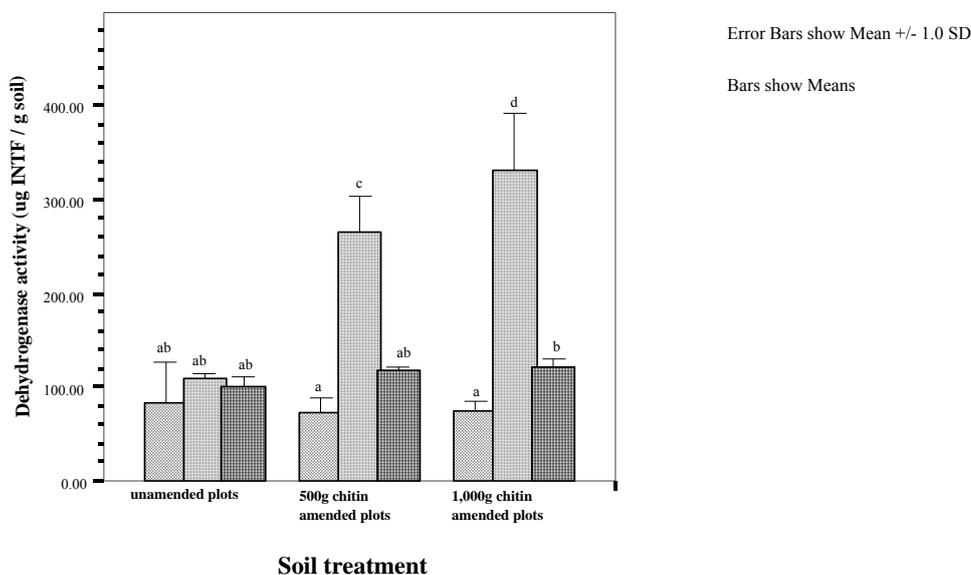


Figure 1. Dehydrogenase activities of the soil measured at one week before transplanting, four weeks after transplanting and one week after harvesting from tomato planting field plots amended with chitin at the rate of 500 g and 1,000 g per plot (1x6 m) and unamended control plots. Means followed by different letters indicate significant differences at $p < 0.05$ from an average of three replicates.

Chitinase activity

The chitinase activity (μg PNP released/g soil) of the soil measured from chitin amended tomato field plots and unamended control was illustrated in Figure 2. Soil chitinase activity values at 4 weeks after tomato transplanting of the 1,000g chitin amended plots, 500g chitin amended plots and unamended plots were increased 1.86, 0.812 and 0.47 times, respectively, higher than the values measured one week before transplanting. While the increasing rates of chitinase activity of 0.81, 0.33 and 0.03 times from the 1,000g chitin amended plots, 500g chitin amended plots and unamended plots were still obtained at one week after harvesting. Sustainable chitinase activity, was therefore increased by chitin amendment application as indicated by the measured value of this last period of time.

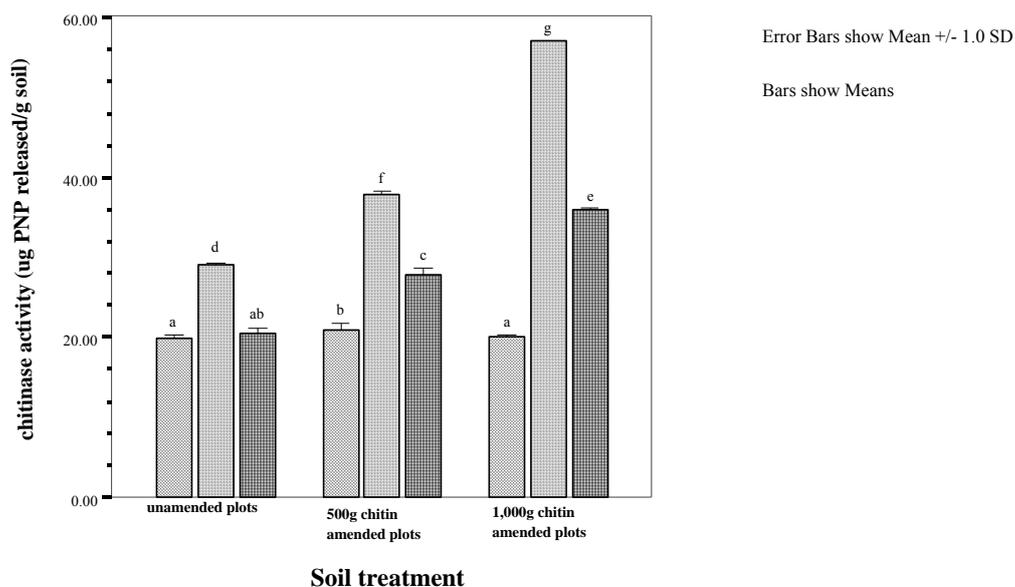


Figure 2. Chitinase activities of the soil measured at one week before transplanting, four weeks after transplanting and one week after harvesting from tomato planting field plots amended with chitin at the rate of 500 g and 1,000 g per plot (1x6 m) and unamended control plots. Means followed by different letters indicate significant differences at $p < 0.05$ from an average of three replicates.

Flourescein diacetate (FDA) hydrolysis of the total microbial activity

Flourescein diacetate (FDA) hydrolysis assay was employed for the measurement of total microbial metabolic activity in the experimental soil plots. The FDA hydrolysis rates were significantly greater in soil receiving input of chitin than unamended tomato field plots (Figure 3). The highest total microbial activity was obtained in 1,000g chitin amended tomato field plots, where the FDA hydrolysis amount expressed as μg flourescein released/g soil was significantly increased up to 1.2 times from the values measured one week before transplanting within 4 weeks after tomato transplanting. While the FDA values were increased 0.7 times in 500g chitin amended plot and 0.2 times in unamended soil plots at this 4 week after transplanting period. A high FDA value was still obtained at one week after harvesting in 1,000g chitin amended plot with 0.7 times higher than the values measured at one week before tomato transplanting. Total microbial activity was also remained in 500g chitin amended plots and unamended plots at one week after harvesting with lesser increasing rate of 0.19 and 0.014 times from the original soil plots, respectively.

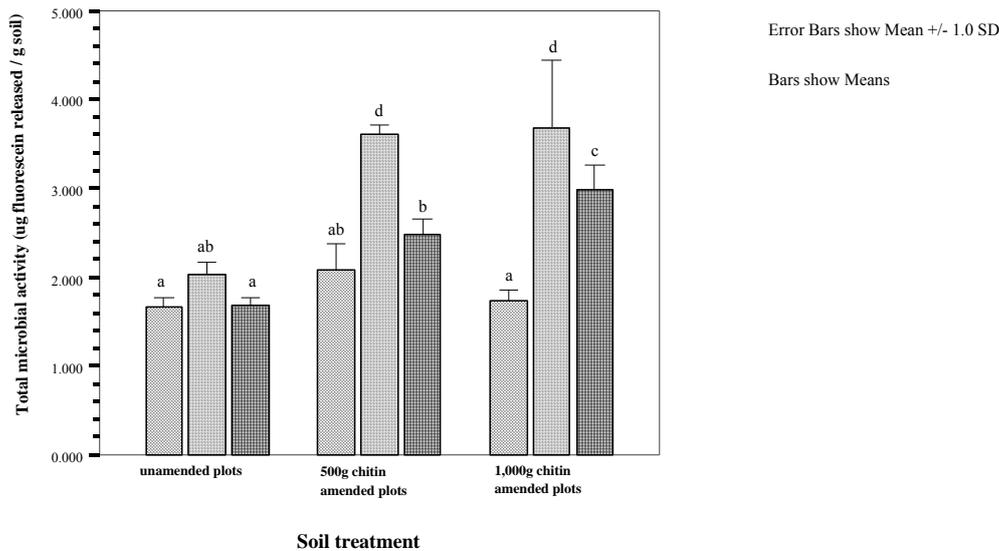


Figure 3. Total microbial activities as defined by FDA hydrolysis of the soil at one week before transplanting, four weeks after transplanting and one week after harvesting from tomato planting field plots amended with chitin at the rate of 500 g and 1,000 g per plot (1 x 6m) and unamended control plots. Means followed by different letters indicate significant differences at $p < 0.05$ from an average of three replicates.

Discussion

A higher level of dehydrogenase activity was observed in chitin amended sandy soil tomato field plots than in the control plots. The increasing levels of dehydrogenase activity amount was observed within 4 weeks after tomato transplanting. Although this activity increased also in unamended soil plots but with much lower level. Significant increasing rates up to 2.6 – 3.5 times were obtained in 500-1,000g chitin amended plots while only 0.32 times were from unamended plots at this 4 weeks after transplanting period. The soil dehydrogenase activity was gradually decreased after tomato plants had been removed as seen by the values measured at one week after harvesting (Figure 1). The results indicate the proliferation of active soil microbial primary metabolism and thus the proliferate amount of soil aerobic microorganisms induced by chitin amendment. As it has been demonstrated by several researchers that the total range of oxidative activity of soil microflora and also a cellular aerobic respiratory process are reflected by the soil dehydrogenase activity (Lenhard, 1956; Skujins, 1976; Maurines-Carbonell et al., 1989; Rossel et al., 1997.). The action of chitin amendment application on dehydrogenase activity in this observation was quite similar to the action of several other organic amendment fertilizers such as manure, compost and vermicompost. There have been reported in

Many cases that organic amendment of the soil can markedly increase the dehydrogenase activity as demonstrated by Perucci (1992), Martens et al. (1992), Serra-Wittling et al. (1995), Pascaual et al. (1997), Masciandaro et al. (1997), Marinari et al. (2000), Lee (2004), Arancon et al. (2006) and etc. The dehydrogenase activity could have been increased by an average 2-4 fold in organic amended soil than the unamended soil (Marten et al., 1992). Approximately 4-20 times higher dehydrogenase activity was achieved from the food waste compost amendment than that of unamended and mineral fertilizer treatments (Lee et al., 2004.). These results were

quite similar to our chitin amendment application that about 2.64- 3.46 times higher dehydrogenase activity than unamended tomato field soil plots.

In the case of soil chitinase activity, interesting increasing pattern was observed (Figure 2). The chitinase activity level of the soil from 500g and 1,000g chitin amended tomato field plots increased 0.81 and 1.86 times within 4 weeks after transplanting from the starting level measured at one week before transplanting and the activity was retained 0.33 and 0.81 times, respectively, in this treated soil plots at one week after tomato harvesting. While the level of chitinase activity of unamended plots increased 0.47 times in the fourth week after transplanting and rapidly declined with only 0.03 times higher than the level at one week before transplanting. These results suggest that the soil chitinase activity is effectively elevated at to a high level then can it be sustained over a period after tomato harvesting which is more than 12 weeks by chitin amendment application. The highest chitinase activity was also attained by the amendment with chitin powder in the comparative study of inductive chitinolytic enzymes production among chitin, other carbon sources and chitinase producing *Streptomyces* (Ueno and Miyashita, 2000). Similarly, assessment of chitin decomposer diversity within upland grassland had shown that the chitinase activity was highly increased by chitin amendment (Krsek and Wellington, 2001). Degradation of chitin in soil by chitinolytic microorganisms was investigated and revealed a complete decomposes process within 16 weeks (Hirano et al., 1991; De Boer, et al. 1999). More than half of the chitin had been decomposed after 4 weeks of incubation in dune soils and hence successfully activated the growth and reproduction of chitinolytic microorganisms including fungi, actinomycetes and unicellular bacteria (Gray and Baxby, 1968; Gould et al., 1981; De Boer, et al. 1999). Buried chitin in sand dunes was also degraded within 8 weeks and the mean recovery of chitin N was 51% after 8 weeks and 57% after 16 weeks of incubation period (Hirano et al. 1991.). Thus, the chitin left over residues from the amendment application in our experiments could be responsible for a remaining high chitinase activity of the soil microorganisms through one week after harvesting or even longer period of time. So the promotion of the plant growth and reduction of plant pathogens in soil could also be subsequently achieved through a period of tomato planting as those has been studied in the case of biological control by the use of chitin (Mitchell and Alexander, 1962; Sarathchandra et al. 1999).

Similar dynamic trend was also revealed in the determination of total soil microbial activity as shown in Figure 3 by a reference standard fluorescein diacetate (FDA) hydrolysis. The FDA hydrolysis was chosen as an appropriate indirect measure of metabolic activity of microbial population in soil because of its simplicity and superb ability that is capable for both intact microorganisms and extracellular enzymes released. (Schnürer and Rosswall, 1982; Taylor and May, 1995 Adam and Duncan, 2001). Total soil microbial activity was increased up to 1.2 times by the amendment of chitin at 1,000 g/plot within 4 weeks after tomato transplanting and stayed at 0.7 times in the last measurement at one week after harvesting. A long

Lasting effect was resembled in these corresponding periods with the use of 500g chitin at slightly lower increasing rate of 0.73 times and later 0.19 times. While the unamended control plots yielded only 0.22 times or 22% increasing on the fourth week of transplanting and 0.014 or 1.4% increasing rate at one week after harvesting. It seemed that the total microbial activity was activated only during the growth of tomatoes in unamended plots and rapidly declined upon harvesting period. This occurrence is commonly found in many vegetation soils that the total microbial activity has been increased due to the development of vegetation and will be declined soon afterward (Long et al., 2005). A transient effect was similarly displayed in the case of sewage sludge and household organic waste compost amendment and in the study of organic matter decomposition (Debosz et al., 2002; Hiroki et al., 2007). A work on organic

matter amendment by Pankhurst et al. (2005) showed that incorporation of organic amendments into a sugarcane soil including chitin initially increased fungal and bacterial populations, microbial activity (FDA hydrolysis) and microbial biomass with a minor effect on the soils capacity to suppress soil organisms associated with yield decline of sugarcane. Recently, significant increasing of soil microbial activity was withstood by the amendment of shrimp and crab shell powder at 0.5-1% (w/w). In a parallel manner, this type of amendment in the pathogen-infested soil was the most effective in reducing population density of *F. oxysporum* f. sp. *tracheiphilum* and also promoted seedling growth and formation of nodules of asparagus bean (Ha and Huang, 2007). The shell of shrimp and crab is composed mainly of chitin impregnated with protein and mineral salts including minor amounts of magnesium, phosphate, silica, and sulfur. The results obtained from the amendment of this shrimp and crab shell powder, thus could be corresponded to their main component chitin as well. Chitin is known for its highly biodegradable substrate that provide a steady release of essential carbon, nitrogen and a long term source of organic acids and ultimately hydrogen for beneficial microorganisms in soil as has been demonstrated in many cases (Okafor, 1966; Gooday, 1990; Sarathchandra et al, 1999). In our trial, sustaining effect was achieved by the amendment of chitin that the microbial activity had been prolonged to a certain period after harvesting. The increasing rate of microbial activity (4-6 times higher in chitin amendment than unamended control) during tomato growth and quite a long term release source of nutrient holding chitin are possibly responsible for such phenomenon.

In conclusion, the overall results of this present study indicates a stimulation of microbial metabolic activities by 500-1,000g chitin amendment to sandy soil tomato field plots during the growing period. Data obtained from the three assays correlated with a similar dynamic trend. The enhancement of enzyme activities (dehydrogenase, chitinase and FDA hydrolysis) was highest at 4 weeks after tomato transplanting. These results suggest a strong active microbial activities in tomato rhizosphere was stimulated by a chitin supplement. These enzyme activities were gradually declined after harvesting period (which was about 12-13 weeks since the chitin was first added to soils). However the remaining levels of activities were still much higher than unamended control plots.

Acknowledgements

The authors gratefully acknowledge Khon Kaen University for research funding support.

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