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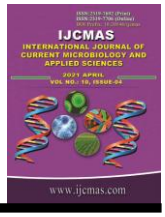

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Evaluation of Pesticides on Dehydrogenase, Urease and Phosphatase Enzymes Activities in A Tropical Red Soil under Cabbage Cropping

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A B S T R A C T

Occurrence of high density of pest infestation at different growth stages of cabbage results into severe yield reduction. Innumerable inorganic and organic pesticides are used to control pests; however it negatively affects soil enzymes. Five pest management schedules viz. FYM + RDF + pest management as per package of practice (PMPP), FYM + RDF + integrated pest management (IPM), FYM + RDF + organic pesticides, organic nutrients + organic pesticides (control) and FYM + RDF + pest management followed by farmers (PMF) were evaluated to assess the impacts of these pesticide residues on dehydrogenase, urease and phosphatase enzymes activity in soil under cabbage cropping. Results showed that inhibition of enzyme activity was mostly observed at high amounts of pesticides, but promotion or stimulation of enzyme activity was observed at low amounts. Highest inhibition of dehydrogenase activity (30.63%) was observed in soil with higher concentration of pesticides with the treatment of FYM + RDF + PMF. Increased dehydrogenase enzyme activity (2.30%) was noticed in soil treated with FYM + RDF + organic pesticides as compared to control. Dehydrogenase activity showed highest sensitivity to pesticides followed by urease and phosphatase whereas maximum stimulation was recorded with phosphatase. Treatment of FYM + RDF + IPM was found the best for augmenting soil enzymes activities, and thus advisable to the farmers for sustainable production of cabbage grown in tropical red soil.

Introduction

In modern explosive agriculture, various chemical fertilizers and pesticides have been used extensively for maximizing crop production to meet the food demand of the rising human population. Pesticides constitute both organic and inorganic agrochemicals of diversified chemical compositions. The judicious application of these chemicals is beneficial for sustaining the soil and crop productivity through controlling pest infestation in soil and plant system. However, the prolonged and indiscriminate use of the

pesticides cause harmful effects on soil health, environmental pollution, food chain contamination and disrupt the microbial diversity and biological functions in soil (Ejah *et al.,* 2004; Mahía *et al.,* 2008; Muñoz- Leoz *et al.,* 2013). Soil enzymes driven by microorganisms are the key indicators for several biochemical processes in soil (Caldwell, 2005; Riah *et al.,* 2014). They play an important role in mineralization and transformation of organic matter and nutrient recycling in soil, energy transformation, stability in soil structure, nitrogen fixation, disease control, and other biochemical transformations such as ammonification, nitrification and phosphorus solubilization leading to increased vigour, growth and productivity of crop (Prasad Reddy *et al.,* 1984; Husain *et al.,* 2003; Karas *et al.,* 2018; Jat *et al.,* 2021).

Soil dehydrogenase is a potential intracellular enzyme in all living microbial cells. It is used for assessing the microbial oxidative activity in soil and a potential measure of microbial biomass and soil respiration (Subhani *et al.,* 2001; Zhang *et al.,* 2016). Dehydrogenase enzyme is considered to play a significant role in the biological oxidation of soil organic matter by donating hydrogen from organic substrates to inorganic acceptors (Zhang *et al.,* 2010; Moeskops *et al.,* 2009). It is originated from several sources like bacteria, fungi, protozoa and plant roots and can serve to transform the complex and even unavailable forms of organic P into inorganic available P forms through catalytic hydrolysis in the soil, which is subsequently absorbed by plants and microbes (Dick and Tabatabai, 1993; Hinsinger, 2001). Soil phosphatase activity is a potential detector of mineralization of organic phosphorus and biological activity of soils. It maintains and controls the rate of phosphorus cycling through soils, particularly in high phosphorus deficient organic soils (Chen *et al.,* 1996). Acid phosphatase activity in general was found predominant in acid soils while alkaline phosphatase activity in neutral or alkaline soils (Dick *et al.,* 2000). Addition of pesticides or inorganic P in soil can inhibit the enzymatic activities involved in the phosphorus cycle (Kalam *et al.,* 2004). Soil urease is one of most active hydrolytic enzymes in soil and a key component in soil nitrogen cycle. It is derived mainly from plants and microorganisms and does exist both as intra- and extra-cellular enzyme (Riah *et al.,* 2014). It catalyses the hydrolysis of applied urea into carbon dioxide and ammonia which is taken up by plants. Application of pesticides decreased activity of urease enzyme in soil due to reduced urea hydrolysis, which is beneficial, because it helps to maintain nitrogen availability to plants for longer period (Antonious, 2003). The urease activity in soil is either unaffected or inhibited by addition of pesticides depending upon the balanced soil fertilization and management practices.

Cabbage is one of the major vegetable crops in temperate and tropical climates of India. It is grown extensively during winter as well as summer season due to its wide adaptability. However, the crop is susceptible to several insect pest infestations in the field, which causes heavy economic losses to the farmers (Baidoo and Mochiah, 2016). Broad-spectrum of synthetic pesticides is applied to effectively control the pests. Among the vegetables in India, cabbage is the maximum pesticide-consuming crop. Moreover, pesticides have also entered into plant system causing more chance of pesticide intake to humans and animals. Many agricultural management strategies have been evolved to minimize the use of pesticides, soil health promotion and yield sustainability. There are innumerable evidences showed that different pesticides have inhibitory and/ or promotory effects on soil enzymes which is largely governed by chemical and recalcitrant nature of the pesticides, physical and chemical properties of

soils and cropping conditions (Kiss *et al.*, 1975). Keeping the above considerations in view, the present study was conducted to assess the impacts of different management schedules of pesticides on dehydrogenase, urease and phosphatase enzymes activity in a red soil of northern transition zone of Karnataka under cabbage cropping.

Materials and Methods

The field experiment was conducted during the summer season (March-June) of 2015-16 on cabbage in a red soil of northern transition zone of Karnataka belonging to the tropical climate of south India. The experimental site is located at $15^0$26" N latitude and $75^0$07" E longitude at an altitude of 678 m above mean sea level. The soil is sandy clay loam in texture.

Cabbage (*Brassica oleracea var*. capitata) cv. Golden head N50 was used as test crop. Five weeks old healthy seedlings were transplanted in the well prepared main field with crop geometry of 45 cm row to row and plant to plant distances. Farmyard manure @ 25 t ha^{-1} and recommended dose of fertilizer (N:P:K::150:100:125 kg ha^{-1}) in the form of urea, single superphosphate and muriate of potash, respectively were applied as per adopted treatments. FYM including half dose of N and full dose of P and K were applied as basal during the final land preparation and remaining half dose of N was top-dressed at 45 days after transplanting. In organic nutrient schedule, only FYM was used as sources of nutrients. The crop was exposed to five types of pest management practices viz., T_1: FYM + RDF + pest management as per package of practice (PMPP), T_2: FYM + RDF + integrated pest management (IPM), T_3: FYM + RDF + organic source of pesticides, T_4: organic sources of nutrients + organic sources of pesticides (considered as control) and T_5: FYM + RDF + pest management usually followed by farmers (PMF). The scheduling of different doses of plant protection chemicals against pest attack during the cropping period followed is given in Table 1 and Table 2.

The routine intercultural operations like gap filling and manual weeding were followed uniformly in each sub-plot. The relevant physical and physicochemical characteristics of the initial experimental soil (0-15 cm) as determined by the standard methods (Piper, 1996; Jackson, 1973) are furnished in Table 3.

The treatment-wise and replication-wise composite soil samples (0-15 cm) were collected at harvest, processed, moistened to 60% of maximum water holding capacity (field capacity) by adding requisite amount of distilled water and pre-incubated at 37^0C in a BOD incubator for a period of 1-day for urease and 7-day for phosphatase and dehydrogenase enzymes (Basavaraj, 1984). A known quantity of pre-incubated soil (5 g for dehydrogenase, 1 g for phosphatase and 10 g for urease activity) was placed in glass tubes and brought to field capacity. The tubes were covered with rubber stoppers and were incubated in a BOD (Biological Oxygen Demand) incubator at a temperature of 37^0C in order to restore the normal biological activity. Phosphatase activity was determined by quantifying P-nitrophenol hydrolyzed from P-nitro phenol phosphate used as a substrate (Tabatabi, 1982). Urease activity was determined by quantifying unhydrolysed urea from which amount of urea hydrolyzed was computed (Watts and Chrisp, 1954). Dehydrogenase activity was estimated by quantifying triphenyl formazan (TPF) formed from 2,3,5-triphenyl tetrazolium chloride (TTC) (Casida *et al.*, 1964). The per cent inhibition or promotion of enzymes activity due to various chemicals applied in cabbage field is calculated as, $(s - t) \times 100/s$ where, s = the enzyme activity value for control and t = the enzyme activity value for treatments.

The data obtained for different treatments were subjected to analysis of variance (ANOVA) using Microsoft Excel 2016 and SPSS version 23.0 (SPSS, Inc.). Fisher's least significant difference (LSD) test was employed and treatments means were separated by Duncan Multiple Range Test (DMRT) at P <0.05 level (Gomez and Gomez, 1984).

Results and Discussion

Effect of pesticides on soil dehydrogenase activity

The changes in dehydrogenase activity were significantly affected by the application of pestiticides (Table 4). Highest significant reduction of dehydrogenase activity in comparison with control (T_4) was observed in T_5 (5.73 µg TPF g^{-1} soil hr^{-1}**)** where fertilizers and manures as per recommendation and pesticides as per farmers' practice were applied. This reduction was followed by T_2 (7.95 µg TPF g^{-1} soil hr^{-1}**)** where RDF and FYM were incorporated as per package of practices and pest control through integrated pest management and T_1 (7.85 µg TPF g^{-1} soil hr^{-1}**)** where nutrients and pesticides were applied as per package of practices. The latter two treatments (T_1 and T_2) were statistically at par. Significantly highest dehydrogenase activity as compared to control was found with T_3 (8.45 µg TPF g^{-1} soil hr^{-1}**)** where RDF and FYM were administered along with organic pesticides. The control treatment T_4 constituting only organic sources of nutrients as well as pesticides showed the dehydrogenase activity of 8.26 µg TPF g^{-1} soil hr^{-1}.

The soil exposure of pesticides application may affect enzymes activities either directly or indirectly. Direct inhibition of intracellular and extracellular enzymes can occur by binding of pesticides to protein molecules in a reversible mode (Browman and Tabatabi, 1978). Indirect inhibition refers to changes in microbial activities and concurrent altered intra- and extracellular enzyme levels (Cervelli *et al.,* 1978). Soil dehydrogenase activity is often used as a measure of the metabolic activity of microorganisms in the soil. Any exogenous toxicant addition to soil may modify the microorganisms and thus dehydrogenase enzymes. Several variations in dehydrogenase activity have been observed in soils with different kinds of pesticides and nutrients. The per cent inhibitory/promotory values for various organic and inorganic treatments on dehydrogenase activity calculated with reference to control are summarized in Table 7.

It is conspicuous from the computed results that the promotion or demotion of dehydrogenase activity in soil was mainly controlled by the kinds of pesticides used for pest management programme. Soils treated with inorganic pesticides showed mostly inhibitory effect on soil dehydrogenase enzyme activity while stimulatory effects in soils treated with organic pesticides when compared with the control providing only organic sources of nutrients and pesticides. The decrease in enzyme activity with increase in concentration and spray schedule was due to the lethal action of applied pesticides on microorganisms, which in turn affected the enzymatic processes. These findings have been supported by Chandrayan and Sethunathan (1980) with HCH and carbaryl; Tu (1981) with trebufos, triazophos and trichlornar and Kalam *et al.,* (2004) with profenofos with suppressed dehydrogenase activity.

Highest inhibition of dehydrogenase activity (30.63%) was observed with higher concentration of pesticides in T_5 comprising of FYM + RDF + pest management followed by farmers. This might be attributed to physical

structure of the chemicals applied which likely have more lethal action to microorganisms releasing dehydrogenase enzymes. Relatively less inhibition of dehydrogenase activity was demonstrated in T_1 (4.96%) and T_2 (3.75%). Inhibition or demotion of dehydrogenase activity in soil was noticed by Purushothaman *et al.,* (1974) for cytrolane, Srimathi *et al.,* (1986) for bromophos and Nelson and Li (1985) for captan. Mayanglambam *et al.,* (2005) recorded 35.5% inhibition of dehydrogenase activity by application of quinalphos which almost similar to our findings. Topal *et al.,* (2014) revealed that acute exposure to chlorpyrifos showed time dependent decrease in glucose-6-phosphate dehydrogenase enzyme activity at all concentrations. Sanchez-Hernandez *et al.,* (2017) also recorded 47% reduction of dehydrogenase activity as compared to control.

Increased dehydrogenase enzyme activity (2.3%) over control was only observed in soil treated with FYM + RDF + organic sources of pesticides (T_3) which could be attributed to increased availability of soil nutrients used by microorganisms releasing dehydrogenase enzymes. This observation is in line with Manna and Ganguly (2001) that incorporation of FYM and N-fertilizer increased the soil microbial biomass C, N and P. Similarly, Sun *et al.,* (2003) reported long-term application of mineral NPK fertilizer combined with organic manure significantly increased the contents of organic matter, total N, P and available N in soil which resulted in increased soil enzyme activities including dehydrogenase.

Effect of pesticides on soil phosphatase activity

The analytical data on significant impacts of different pesticides on soil phosphatase activity is furnished in Table 5. Maximum inhibition of soil phosphatase activity relative to control treatment (T_4) was observed in T_5 (5.40 µg P-NP g^{-1} soil hr^{-1}) which was followed by T_1 (5.83 µg P-NP g^{-1} soil hr^{-1}), while maximum and marginal promotion of phosphatase activity was found in T_2 (6.30 µg P-NP g^{-1} soil hr^{-1}) and T_3 (6.07 µg P-NP g^{-1} soil hr^{-1}), respectively.

The treatments T_4 and T_3 were statistically at par with each other. The various scheduling of organic and inorganic pesticide in conjunction with FYM + RDF either demoted or promoted the phosphatase activity, but mostly exhibited inhibitory effect at higher concentrations with more number of spraying. These observations were supported by Zhang *et al.,* (2013) who recorded the significant antagonism effects on soil phosphatase at higher concentrations of acetochlor with As (III).

With reference to control, higher inhibitory effect of pesticides on phosphatase activity (10.30%) was found with T_5 accommodating higher nutritional dose and more number of pesticides applications adopted by farmer followed by that of T_1 (3.16%) showing less inhibition effect (Table 7).

This could be ascribed to the toxic actions of insecticides on P-solubilizing population which might have altered the membrane permeability of the microorganisms producing phosphatase enzymes.

In contrast, Voets *et al.,* (1974) recorded 61.8% inhibition of the phosphatase activity in forest soil as a result of atrazine application. Similar but rather increasing trends were noticed by Tabatabai and Bremner (1969) for p-nitrophenyl phosphate, Krishnamurthy (1989) for fenvelerate, Madhuri and Rangaswamy (2002) for dichlorvos, phorate and methomyl, Kalam *et al.,* (2004) for propiconazole, Kennedy and Arathan (2004) for carbofuran and Sanchez-Hernandez *et al.,* (2017) for chlorpyrifos.

Table.1 Pesticide application pattern

Treatment	15 DAS at Nursery	15 DAT	30 DAT	45 DAT	60 DAT	75 DAT
T_1	Dimethoate	Dimethoate	Dimethoate	Malathion	Malathion	Malathion
T_2	Dimethoate	Neem oil	Neem oil	Neem oil	Dimethoate	Neem oil
T_3	Dimethoate	Neem oil	Neem oil	Neem oil	Neem oil	Neem oil
T_4	Dimethoate	Neem oil	Neem oil	Neem oil	Neem oil	Neem oil
T_5	Dimethoate	Endosulfan	Dimethoate	Dimethoate	Indoxacarb	Indoxacarb

Table.2 Dosages of pesticide application

Chemical used	Dosage
Endosulfan 35 EC	2 ml L^{-1}
Dimethoate	2 ml L^{-1}
Neem oil	2 ml L^{-1}
Indoxacarb 14.5 SC	5 ml 10 L^{-1}
Malathion	2 g L^{-1}

Table.3 Physicochemical characteristics of the experimental soil

Soil properties	Value
Clay (%)	35.2
Silt (%)	7.6
Fine sand (%)	29.2
Coarse Sand (%)	28.0
Texture	Sandy clay loam
Water holding capacity (%)	59.1
Organic carbon (g kg^{-1})	2.4
pH	7.4
EC (dS m^{-1})	0.23
CEC [cmol (+) kg^{-1}]	33.0

Soil pH and EC determined in 1:2.5 soil: water suspension

Table.4 Effect of pesticides on soil dehydrogenase activity

Treatments	**Dehydrogenase activity (µg TPF g^{-1} soil hr^{-1})**
T_1: FYM + RDF + PMPP	7.85 (2.97)[c]
T_2: FYM + RDF + IPM	7.95 (2.99)[c]
T_3: FYM + RDF + Organic pesticides	8.45 (3.07)[a]
T_4: Organic nutrients + Organic pesticides	8.26 (3.04)[b]
T_5: FYM + RDF + PMF	5.73 (2.59)[d]
S.Em.±	0.06
CD (0.05)	0.17

The values in the parenthesis are transformed into $\sqrt{X+1}$; means followed by the same letters in a column are not differed significantly by Dunkan Multiple Range Test ($P < 0.05$); PMPP: pest management as per package of practice, IPM: integrated pest management, PMF: pest management followed by farmers

Table.5 Effect of pesticides on soil phosphatase activity

Treatments	**Phosphatase activity (µg P-NP g^{-1} soil hr^{-1})**
T_1: FYM + RDF + PMPP	5.83 (2.61)[b]
T_2: FYM + RDF + IPM	6.30 (2.70)[a]
T_3: FYM + RDF + Organic pesticides	6.07 (2.66)[ab]
T_4: Organic nutrients + Organic pesticides	6.02 (2.65)[ab]
T_5: FYM + RDF + PMF	5.40 (2.53)[e]
S.Em.±	0.019
CD (0.05)	0.057

The values in the parenthesis are transformed into $\sqrt{X+1}$; means followed by the same letters in a column are not differed significantly by Dunkan Multiple Range Test ($P < 0.05$); PMPP: pest management as per package of practice, IPM: integrated pest management, PMF: pest management followed by farmers

Table.6 Effect of pesticides on soil urease activity

Treatments	**Urease activity (µg urea g^{-1} soil hr^{-1})**
T_1: FYM + RDF + PMPP	259.25 (16.13)[a]
T_2: FYM + RDF + IPM	270.50 (16.48)[a]
T_3: FYM + RDF + Organic pesticides	268.50 (16.41)[a]
T_4: Organic nutrients + Organic pesticides	260.25 (16.16)[a]
T_5: FYM + RDF + PMF	183.13 (13.57)[b]
S.Em.±	0.15
CD (0.05)	0.45

The values in the parenthesis are transformed into $\sqrt{X+1}$; means followed by the same letters in a column are not differed significantly by Dunkan Multiple Range Test ($P < 0.05$); PMPP: pest management as per package of practice, IPM: integrated pest management, PMF: pest management followed by farmers

Table.7 Percentage inhibition or promotion of enzyme activity in soil

Treatments	**Percentage inhibition or promotion**		
	Dehydrogenase activity	**Phosphatase activity**	**Urease activity**
T_1: FYM + RDF + PMPP	4.96	3.16	0.38
T_2: FYM + RDF + IPM	3.75	4.65*	3.94*
T_3: FYM + RDF + Organic pesticides	2.30*	0.83*	3.17*
T_4: Organic nutrients + Organic pesticides	0.00	0.00	0.00
T_5: FYM + RDF + PMF	30.63	10.30	29.63

*indicate promotion; PMPP: pest management as per package of practice, IPM: integrated pest management, PMF: pest management followed by farmers

Treatment receiving FYM + RDF + IPM (T_2) employing minimum use of chemical pesticides showed maximum stimulatory or promotory effect on phosphatase activity (4.65%) as compared to control (Table 7). This increase in soil phosphatase activity might be due to increase in P- solubilizers as a consequence of degradation of applied insecticides or products which might have served as a carbon source. Dimethoate, the only chemical pesticide applied in this treatment, is an organophosphorus group of insecticides which might have provided direct source of P after its degradation. This stimulatory effect of pesticides on P-solubilizers was supported by Sivasithamparam (1969) with chlorpyrifos and Congregado *et al.*, (1979) with organophosphorus insecticides.

Likewise, treatment having FYM + RDF + organic source of pesticides (T_3) also showed minor stimulatory effect on soil phosphatase activity which could be attributed to applied fertilizers and manure serving as sources of nutrients to soil microorganisms releasing phosphatase enzymes in soil. This is in agreement with the works of Gopal Reddy (1997) who reported highest dehydrogenase, urease and phosphatase activity with RDN + FYM as compared to treatment with only inorganic source of RDF. Lalfakzuala *et al.*, (2006) reported that fertilizer treatment increases microbial population number and microbial enzymatic activity. Kondratowicz (2007) also found that fertilization with nitrogen and manure resulted in an increase in microbial population and higher enzymatic activity in soil.

Effect of pesticides on soil urease activity

The impacts of various treatments of pesticides on soil urease activity were at par except in treatment T_5 where fertilizers and manure as per recommendation and pesticides as per farmers' practice adopted, which showed the highest reduction of urease activity (183.13 µg urea g^{-1} soil hr^{-1}) in comparison with control (Table 6).

Highest inhibition on soil urease activity was observed in T_5 (29.63%) with higher nutritional dose and more number of pesticides application followed by farmers. The inhibitory effect of pesticides on urease may be direct or indirect. An indirect effect may result from desorption of the enzyme. The stability of urease increases by sorption on soil; after desorption its activity will be reduced as reported by Lampe and Aldag (1979). The inhibition of urease activity with higher pesticides applications may be attributed to the higher sand content, which may result in lesser sorption of the chemical and also desorption of the urease from the soil colloids. Inhibitory effect on urease activity upon insecticide application was also observed by Lethbridge and Burns (1976) with organophosphorus, Basavaraj (1984) with combined application of 2,4-D, carbofuran and quintozene, Srimathi *et al.*, (1986) with bromophos, Elliot (1989) with fungicides, Kennedy *et al.*, (1999) with carbofuran and Laksmikantha (2000) with fenvalerate, quinalphos and endosulfan.

Treatments T_1, T_2 and T_3 showed relatively higher urease activity than control which might be attributed to the increased microbial population due to addition of fertilizers and FYM as enzyme activity is directly related to the microbial count in soil as reported by Dinesh *et al.*, (2000). The increased urease activity with addition of RDF and FYM was also reported by Sriramachandrasekharan (2002).

The most substantial index of biological activity in soil is its enzymatic activity which can give an idea of the biochemical processes in soil. The enzyme acts as indicator of the

soil quality and fertility. Among three soil enzymes studied, dehydrogenase showed highest sensitivity followed by urease. Lowest reduction in enzymatic activity was observed in case of soil phosphatase which is supported by work of Kawalczyk and Wlike (1989) who observed no significant effect on phosphatase activity by sodium dodecyl benzosulphonate, a formulation agent for various pesticides. Monarik and Malickenko (1969) did not observe much effect of triazines on phosphatase activity in soil. Lewis *et al.,* (1978) also reported the same. Oleszczuk *et al.,* (2014) observed that biochar stimulated the activity of enzymes and it also reduced the negative effect of pesticides on the enzymatic activity and on certain microorganisms in the Microbial Assay for Risk Assessment (MARA). Majumder and Das (2016) reported that insecticides incorporation in soil significantly increased the acid and alkaline phosphatase activities of the soil.

The result of the study showed that soil enzymes activities were inhibited maximum with higher concentrations of inorganic pesticides with the treatment of FYM + RDF + pest management followed by farmers and promoted with the treatment of FYM + RDF + organic source of pesticides. Higher concentrations of chemical pesticides with more number of spraying mostly showed inhibitory effect on soil enzymes, but treatment with FYM + RDF + integrated pest management (IPM) with minimum use of chemical pesticides or pest management with organic pesticides showed stimulatory effect on enzymes activities. Among three soil enzymes studied, dehydrogenase showed highest sensitivity to pesticides applied followed by urease and phosphatase. Considering the efficacy of pesticides on enzyme activity, application of FYM + RDF + IPM recorded was found the best treatment approach. The information generated help to understand the effect of applied pesticides on soil enzymes which are of paramount importance in assessing nutrient recycling and transformation in soil system for sustaining crop productivity.

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