

EFFECTS OF HYPERTHERMIA ON ENZYME PERFORMANCE IN SELECTED PLANTS

ANSHU KAREL*

Department of Botany, S.P.C.Govt. College, Ajmer.

*Corresponding author's email: anshkarel@gmail.com

Adhatoda vasica Nees, *Boerhaavia diffusa* L. and *Parthenium hysterophorus* L are common weeds of mixed habitats growing throughout the state of Rajasthan. Although higher plants develop their own defense strategies to overcome high temperature stress effects, these often are not enough, therefore substantial damage is observed. The metabolism in plants is altered in response to high temperature stress. Among the abiotic stresses, high temperature stress is one of the most detrimental stresses threatening higher plant productivity and survival throughout the world. Of all the enzymes studied in these three weeds, after PRO, catalase was found to be most tolerant to high temperature treatment. In general, both GOT and GPT were also found to be relatively thermo stable. GOT and GPT enzymes of *Adhatoda* sp. exhibited comparatively better thermo tolerance than those of *Boerhaavia* sp. and *Parthenium* sp. In response to climatic changes, weeds pose challenges to crop plants. Further studies on the interactive relations between the two could give functional solutions leading to yield enhancement of crop plants in the proceeding decades.

Keywords: Catalase, crop plants, GOT, GPT, high temperature stress, thermo stable, weeds

Introduction

Temperature is one of the major factors determining the natural distribution of plants. Plants growing in diverging habitats differ in their tolerance to temperatures, ranging from freezing to over 60°C. The stresses in the natural surroundings of plants lead to various physical and chemical interactions, which result in a loss of different functions. Many features in plants allow them to live under a wide temperature range, through phenotypic modifications. These modifications and adaptations help them to mitigate stresses. High temperature stresses above the optimum range are regarded as chronic and acute exposures depending on their time duration. The injuries resulting from these vary to a great extent¹⁻³.

High temperature (HT) means temperatures

so high to damage metabolic processes as a result of which the growth and developmental processes of plants is affected⁴. HT stress is one of the most deleterious abiotic stresses, unfavorable for higher plant productivity and survival throughout the world. In comparison to the 1980–2000 average, temperatures are expected to increase from 1.8 to 4.0 °C or higher by 2100, in accordance to the global climate change scenario⁵. Each degree Celsius increase of the average growing season temperature may decrease crop yield up to 17 % and affect plant distribution. Threatened by adverse effects of HT stresses, plants at lower altitudes will escape towards higher altitudes⁶⁻⁷. HT severely reduces the yield of cultivated and wild plant species⁸. Extreme temperature stress accelerates the generation and reactions of

ROS [such as, singlet oxygen ($^1\text{O}_2$), superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^\cdot)], thereby inducing oxidative stress⁹⁻¹⁰. Mild HT reduces cell expansion and division; on the other hand, severe HT results in programmed cell death. Visual HT damage symptoms include scorching of leaves and twigs, leaf senescence and abscission. Delayed seed germination, atypical seedling development, reduced and altered vegetative growth pattern, male sterility, infertile female gametophyte, abortion of fertilized embryos, fruit abscission, distorted fruit and a loss of vigor are common consequences of HT stress. Also, physiological processes such as water and nutrient transportation, photosynthesis, respiration, and transpiration are affected by HT stress¹¹⁻²⁵.

The pattern of HT effects on plants depends on the temperature factor and plant factor. The temperature factor may include the degree and duration of HT stress, and frequency of temperature exposure. Variables towards the susceptibility to HT in plants are developmental stages, species, genotype and inter- and intraspecific variations of plants^{1-3,26-27}. This paper summarizes the altered enzymatic activities in higher plants (weeds) as a response to HT stress at different levels.

Materials and Methods

The present study was made on following plants, which are fairly common weeds in mixed habitats throughout Rajasthan:

Adhatoda vasica Nees- family Acanthaceae,,
Boerhaavia diffusa L. - family
 Nyctaginaceae and *Parthenium*
hysterophorus L.-family Asteraceae.

Thermal treatment was given and observations were taken at regular intervals to study the effect of high temperature on enzymatic activities.

Extraction of enzyme:

200 mg of fresh sample was homogenized in

10 ml. of 0.2M Tris- HCl buffer (pH 7.0). The extract was centrifuged for 30 minutes at 10,000 rpm. The supernatant was stored in an ice bath and used as source of enzyme.

Enzyme assay:

Catalase activity was measured manometrically²⁸. Aspartate and Alanine aminotransferases commonly known as Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) respectively were assayed²⁹. Plant tissues were extracted in Tris buffer, pH 7.0. The substrate for Alanine aminotransferase was prepared by adding 30 mg of 2-oxoglutarate and 1.78 g of DL-alanine in 100 ml 0.1 M phosphate buffer, pH 7.4. The substrate for Aspartate aminotransferase contained 1.3 g of L- aspartic acid instead of DL-alanine. The pH of the substrate 4, was adjusted by 0.4 N NaOH, if required. The reaction mixture containing 0.5 ml of buffered substrate and 0.1 ml of enzyme extract was incubated for 45 minutes at 37°C. The reaction was terminated by adding 0.5 ml of ketone reagent (20 mg 2,4- dinitrophenyl hydrazine in 100 ml of 1N HCl) and kept at room temperature for 20 minutes. Thereafter, 5.0 ml of 0.4N NaOH was added and the absorbance was recorded at 520 nm against the blank in which 0.1 ml of isolation medium was added instead of the enzyme extract.

Results and Discussion

Catalase:

In vitro: Of all the enzymes studied, after PRO, catalase was found to be the most tolerant to high temperature treatment. Thus, after incubation at 45°C and 50°C for 1 hour 60% activity of this enzyme was still intact in all the three plants (Fig. 1). However, a rapid loss of activity was observed at higher temperatures (55°C and 60°C).

In general, *Parthenium* sp. exhibited better stability than *Boerhaavia* sp. and *Adhatoda* sp. Total loss of catalase activity was observed at 60°C after 45 minutes of treatment.

In vivo: No measurable change in catalase activity could be observed at 55°C upto 1 hour in all the plants. Even after 2 hours of treatment the loss in enzyme activity was only 15% in *Boerhaavia* sp., 20% in *Adhatoda* sp., and 5% in *Parthenium* sp. (Fig. 2). After 4 hours of treatment in all the plants significant activity was still recordable.

Glutamate oxaloacetate transaminase:

In vitro: In all the plants, GOT exhibited a good degree of thermal stability at 45° C (Fig. 3). After incubation at 45° C for 60 minutes, about 70% of control activity was still found intact. *Parthenium* sp. appeared to possess comparatively higher thermal stability as compared to *Boerhaavia* sp. and *Adhatoda* sp.

At 50° C also, very little inactivation of GOT took place up to 30 minutes. Thereafter, the enzyme showed considerable decay. In *Boerhaavia* sp. and *Adhatoda* sp. 50% activity was lost, while *Parthenium* sp. still exhibited comparatively higher activity. The most rapid inactivation of enzymes occurred at 55 °C. Even an exposure for 15 minutes led to 50% inactivation in *Boerhaavia* sp. and *Adhatoda* sp. while in *Parthenium* sp. only 35% decrease in activity was recorded. An almost complete loss of activity after 60 minutes of treatment occurred in all the plants.

In vivo: Treatment at 55° C for 1 hour resulted in a very little change in vivo activity of GOT in *Parthenium* sp., while 25% loss of enzyme activity was perceptible in *Boerhaavia* sp. and *Adhatoda* sp.(Fig. 4).

With an increase in the duration of treatment the enzyme activity showed continuous loss, but the reduction was not severe. This is evident from the fact that even after 4 hours of treatment at 55°C more than 60% enzyme activity was still intact in leaves of all the plants.

At 58°C a rapid inactivation of enzyme was clearly visible. The effect was particularly drastic in *Adhatoda* sp. However, a complete

inactivation could not be observed in any plant even after 4 hours of treatment.

Glutamate Pyruvate Transaminase:

In vitro: At 45° C, GPT showed little change upto 15 minutes of exposure. However a rapid denaturation was observed after 45 minutes (Fig. 5). Incubation at 45° C for 1 hour led to loss of about 60% of control activity in *Boerhaavia* sp. and *Adhatoda* sp., while in *Parthenium* sp. around 50% of control activity was found intact. The progressive denaturation of the enzyme was evident in *Adhatoda* sp. even after 15 and 30 minutes of treatment. On the other hand, in *Parthenium* sp. and *Boerhaavia* sp. significant reduction occurred after 45 minutes of treatment. At 50° C, although the decay in enzyme activity was more pronounced, but a complete loss of activity could not be observed in any plant even after exposure for 1 hour. At this temperature also, *Parthenium* sp. exhibited comparatively less inactivation than *Boerhaavia* sp. and *Adhatoda* sp.

At 55°C, however, a rapid loss of enzyme activity was clearly evident. At this temperature about 65% of control activity was lost within 15 minutes of treatment in all the plants. A complete denaturation of the enzyme, in all the plants, was recorded 1 hour after the treatment.

In vivo: *Parthenium* sp. exhibited fairly good thermal stability even at 55° C for 1 hour, while there was 15% and 30% loss of enzyme activity in *Boerhaavia* sp. and *Adhatoda* sp. respectively. A progressive loss in enzyme activity occurred with an increase in duration of treatment. The loss of activity was found to be more in *Adhatoda* sp. and *Boerhaavia* sp. than in *Parthenium* sp. A complete inactivation did not take place even when the leaves were treated for 4 hours at 55° C. At this time in all the plants around 60% of control activity could still be detected.

At 58° C loss of enzyme activity was more

drastic than at 55° C. However, there were clear differences in thermal tolerance of the three plants. Loss of enzyme activity was more in case of *Boerhaavia* sp. and *Adhatoda* sp. as compared to *Parthenium* sp. (Fig. 6). Again, at this temperature, a complete denaturation could not be observed even after treatment for 4 hours. Higher thermal tolerance of GPT in *Parthenium* sp. was evident throughout the course of the thermal treatment experiments.

Therefore, wide variations were recorded in the thermo stability of different enzymes as studied by in vitro and in vivo experiments. GOT enzyme exhibited fairly good tolerance

to elevated temperatures. In vitro, GOT maintained up to about 70% of control activity at 45° C after a treatment of 1 hour. Complete denaturation of the enzyme was achieved only after 1 hour exposure at 55° C. Similarly, in vivo, GOT showed fairly high thermal tolerance. It did not show complete loss in activity even at 58° C after 4 hours of treatment. In *Typha latifolia* higher thermal stability of GOT than malate dehydrogenase has been reported³⁰. However, because of its narrow amplitude and better thermal tolerance, GOT cannot be important in adaptation to heat stress. In

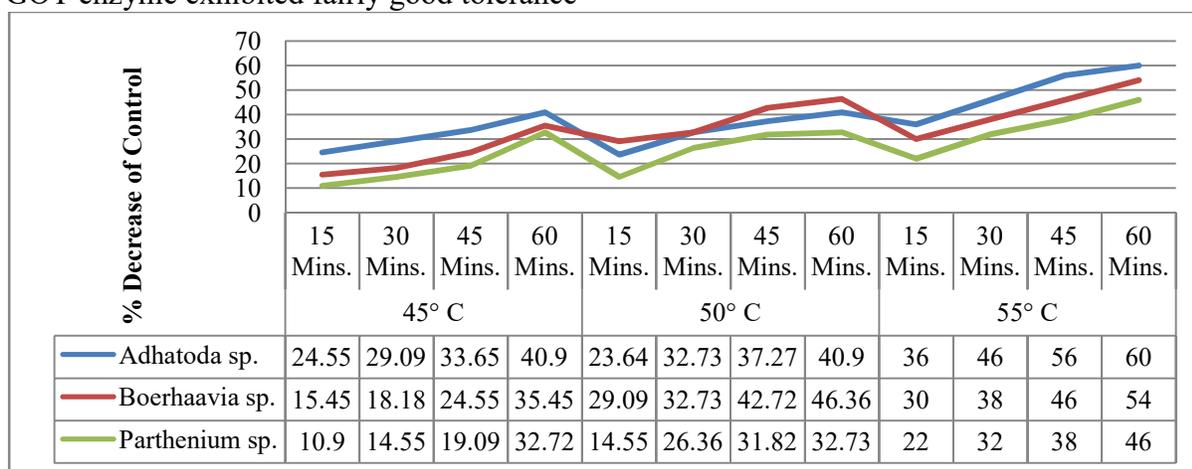


Fig. 1: Effect of Hyperthermia on Catalase *in vitro*

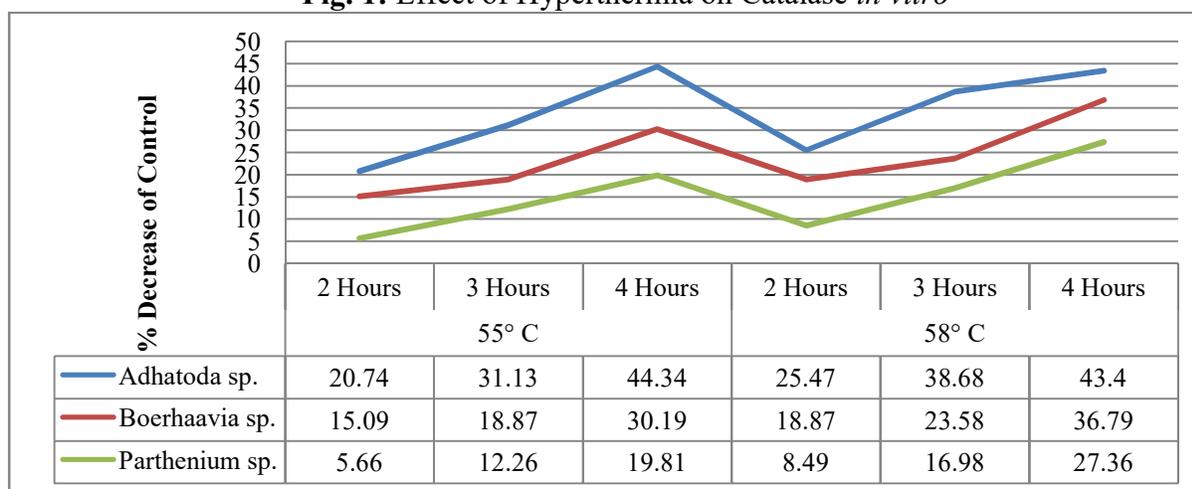


Fig.2: Effect of Hyperthermia on Catalase *in vivo*

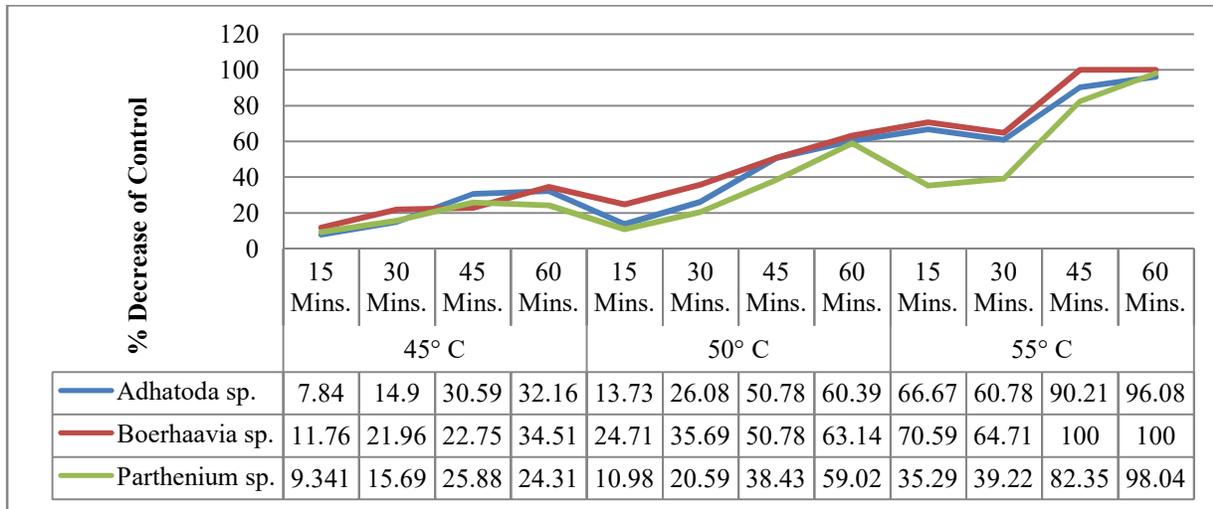


Fig. 3: Effect of Hyperthermia on GOT *in vitro*

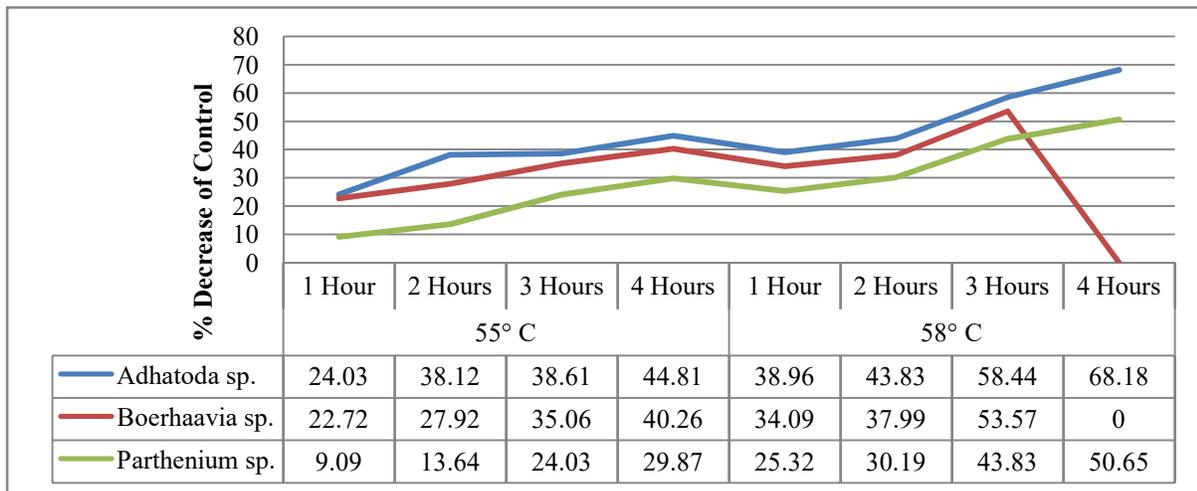


Fig. 4: Effect of Hyperthermia on GOT *in vivo*

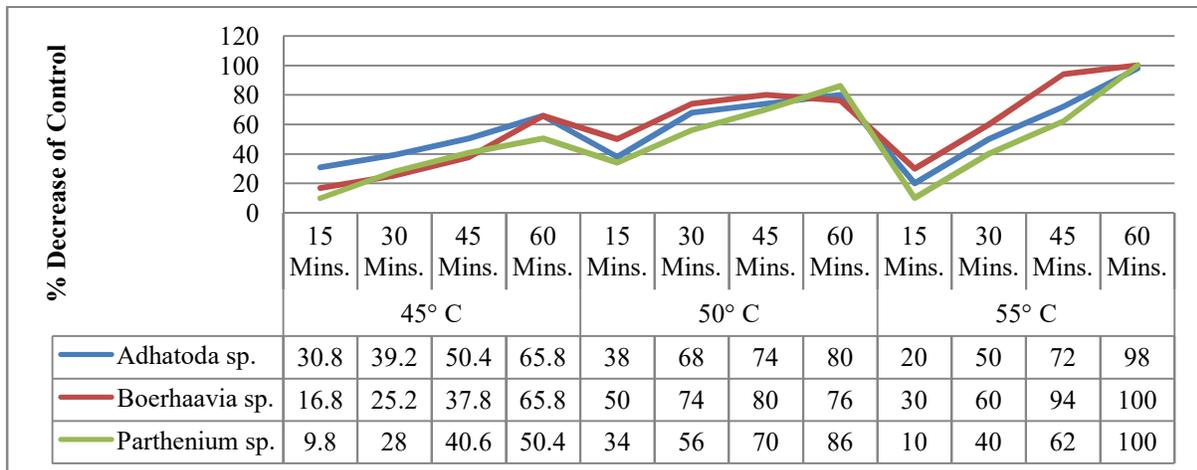


Fig. 5: Effect of Hyperthermia on GPT *in vitro*

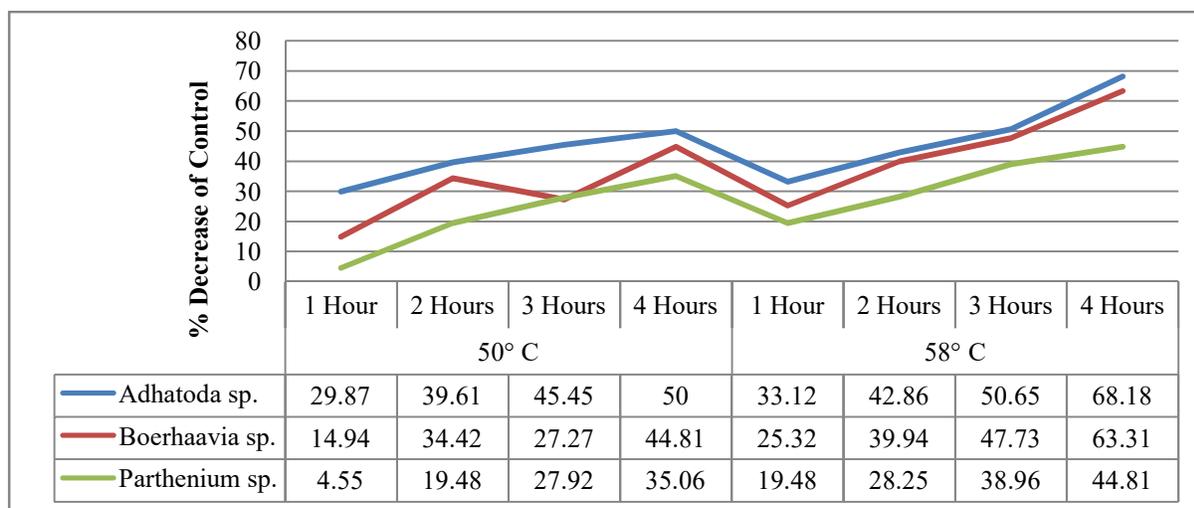


Fig. 6: Effect of Hyperthermia on GPT *in vivo*

comparison to GOT, GPT was found to be comparatively less stable at high temperature. No significant differences in plants with respect to thermal tolerance of this enzyme could be detected.

Most of the heat injuries in plants are supposed to be mediated through inactivation of enzymes³¹. The ability of plants to grow, survive and reproduce successfully under supra-optimal temperatures would largely depend upon thermal tolerance of key enzymes³². In fact, co-relation between thermal tolerance of enzymes and organisms has been found to coexist³³. Notable heat stress effects include structural changes in tissues and cell organelles, disorganization of cell membranes, disturbance of leaf water relations, and impedance of photosynthesis via effects on photochemical and biochemical reactions and photosynthetic membranes. Lipid peroxidation via the production of ROS and changes in antioxidant enzymes and altered pattern of synthesis of primary and secondary metabolites are also of considerable importance. In response to heat stress, plants manifest numerous adaptive changes³⁴. In addition to genetic means to developing plants with improved heat tolerance, attempts have been made to induce heat

tolerance in a range of plant species using different approaches. Although, some progress has been reported as to the development of crop plants with improved heat tolerance by traditional means, there are promising possibilities for engineering plants with heat tolerance. Increased temperature had a greater effect on plant phenological development than elevated CO₂³⁴. Biomass accumulation by annual grass species during their reproductive phase as compared with the vegetative phase were also drastically affected, and such effects were more pronounced in C3 than C4 plant species. The uptake and translocation of herbicides in plants and their persistence in soil were affected by rising temperatures³⁵. In addition, the rate of water absorption and movement, were affected, thereby influencing rate of leaf development, cuticle thickness, stomatal number and aperture. This resulted in indirectly affecting herbicide selectivity and efficacy³⁵⁻³⁶. An increase in atmospheric temperature was found to favor weed growth as well as herbicide efficacy. Although there is a dominance of C4 weeds in agriculture, C3 and C3-C4 intermediate pathways of prominent weeds would pose severe crop-weed competition in the years to come. Importantly, due to species interaction, there

is a need to study all possible combinations of plant-weed carbon fixation pathways while studying the impact of climate change on crop-weed competitive interactions³⁷. Several weeds will exert additional pressure for crop-weed competition under the climate change scenario. More adaptive research studies, including complex research conditions, could yield useful solutions for managing yield reduction in the ensuing decades³⁸.

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