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Alterations in the lactate dehydrogenase activity of the desert locust *Schistocerca gregaria* by the wild plant *Fagonia bruguieri* (Zygophyllaceae).

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ABSTRACT

The wild plant *F. bruguieri* extracts caused some alterations in the lactate dehydrogenase activity in haemolymph and fat body of the last instar nymphs and newly emerged adults of the desert locust *S. gregaria*. Each of two concentration levels of methanolic (7.5 and 3.7%), petroleum ether (30.0 and 15.0%) or n-butanolic extract (30.0 and 15.0%) was applied against the penultimate instar nymphs through the fresh clover leaves, *Medicago sativa*, as a food.

Unexceptionally, all *F. bruguieri* extracts induced the LDH activity in the haemolymph along the nymphal instar, irrespective of the solvent or concentration level. The strongest inducing effect was exhibited in the mid-aged nymphs (62601.3±467.4 U/L in comparison with 53696.8±467.4 U/L of control congeners) after treatment with the higher concentration level of methanolic extract. On the contrary, the LDH activity was significantly prohibited in the newly emerged adults. The most drastically inhibited enzyme activity was determined (57744.3±467.4 U/L, compared to 62871.2±934.7 U/L of control congeners) after nymphal treatment with higher concentration level of n-butanolic extract.

With regard to the fat body, all *F. bruguieri* extracts prohibited LDH activity along the nymphs instar, irrespective of the concentration level. The most dramatically reduced activity was expressed in (9166.4±119.0 U/L vs. 22839.5±289.1 U/L of control congeners) after treatment with the lower concentration level of n-butanolic extract. In contrast, the plant extracts pronouncedly enhanced the enzyme activity in the fat body of adults, regardless to the extract. The most stimulatory effect was exhibited after treatment of the nymphs with the lower concentration level of n-butanolic extract.

Key Words: Lactate dehydrogenase, *Schistocerca gregaria*, *Fagonia bruguieri*, methanol, petroleum ether, n-butanol, nymph, adult.

INTRODUCTION

Pollution of the environment by pesticides is a dangerous problem around the world. Consequently, so many institutions are engaged in some safe alternative methods of pest control. Botanical insecticides and microbial pesticides are highly effective, safe and ecologically acceptable (Matthews, 1999). Several plant and plant-based products are used around the world to control a wide variety of pests. Several plant components responsible for insect control are well-known under the name 'allelochemicals'. Research on the site and mechanism of action of these components indicates that many terpenoid compounds are involved in insecticidal and insect growth regulation activities. These substances are important enzymatic and metabolic inhibitors (Hammond and Kubo, 1999; Cespedes *et al.*, 2000; Kubo *et al.*, 2003;

Panzuto *et al*., 2002). A diverse array of secondary metabolites has different sites of action and different molecular targets when the compounds interact with enzymes and processes of metamorphosis (Calderon *et al*., 2001; Torres *et al*., 2003; Cespedes *et al*., 2004).

Dehydrogenases are very important tools for the investigation of insect metabolic activities during the course of development. The relative activities of the insect dehydrogenases may be related to the function and energy yielding demands of the tissues (Dickinson and Sullivan, 1975).

Lactate dehydrogenase (LDH) (EC 1.1.1.28) is an important glycolytic enzyme that is present in virtually all tissues (Kaplan and Pesce, 1996). It is involved in carbohydrate metabolism and has been used as an indicative criterion of exposure to chemical stress (Diamantino *et al.*, 2001). LDH is, also, a parameter widely used in toxicology and in clinical chemistry to diagnose cell, tissue, and organ damage. However, the potential of this enzyme as an indicative criterion in the invertebrate toxicity tests has been scarcely explored (Ribeiro *et al.*, 1999 ; Senthil Nathan *et al.*, 2006 a & b) . The objective of the present work was to assess the efficacy in the laboratory of different extracts from the wild plant *Fagonia bruguieri* for interfering with the lactate dehydrogenase activity in haemolymph and fat body of the desert locust *Schistocerca gregaria.*

MATERIALS AND METHODS

I) Experimental Insect:

A gregarious stock culture of the desert locust *Schistocerca gregaria* (Forsk.) was raised by a sample from the established culture of Locust and Grasshopper Res. Division, Agric. Res. Center, Giza, Egypt. The insects were reared under crowded breeding conditions outlined by Hunter-Jones (1961) and Hassanein (1965). Newly hatched hoppers were kept in wooden cages with wire-gauze sides (40x40x60 cm) and small door in the upperside to allow the daily feeding and cleaning routine. The bottom was covered with 20 cm layer of sterilized sand. Each cages was equipped internally with 60 W electric bulb for lightening (17:7 LD) and warming (32±2 C.). The relative humidity varied from 70-80% following the introduction of fresh food plant to 60-70% several hours later. Successive generations were raised before obtaining the nymphs for the present experimental work. Fresh food plant was clover *Medicago sativa* along the period of study except few weeks every year because of the absence of this plant species. During these weeks, insects were fed on *Sesbania eagyptiaca*. All experiments were conducted with *M. sativa* only.

II) Plant Extracts:

Fagonia bruguieri var. *bruguieri* is a perennial wild herb distributed all deserts in Egypt but profusely spread in Sinai. It is, also, distributed in Kuwait. Saudi Arabia, Oman, Jordon, Iraq, Palestine, Pakistan, and Afghanistan. It systematically belongs to family Zygophyllaceae. The aerial parts of the plant (leaves, stems and flowers) were collected from the region of Santa Catherin (Sinai) during flowering stage, and kindly identified by Dr. Abdo Marey, Faculty of Science, Al-Azhar University (Cairo). The collected samples were air-dried, powdered and kept in tightly closed amber coloured glass containers for protecting from light, at low temperature.

Dried and pulverized powder of *F. bruguieri* (2 kg) was exhaustively separately extracted with methanol (1.7 Lx3). The combined alcohol extracts were concentrated to 400 ml, diluted with 400 ml of water and the next successively

extracted with petroleum ether (5x400 ml) was concentrated to dryness under reduced pressure giving (80 g), while n-butanol (5x400 ml) extracts were concentrated to dryness under reduced pressure giving (60 g).

III) Nymphal Treatments:

Tow concentration levels of the methanolic extract (7.5 and 3.7%) were used as well as 30.0 and 15.0% of the petroleum ether extract and n-butanolic extract were used.

The newly moulted 4th (penultimate) instar nymphs of *S. gregaria* were fed on fresh leaves of *M. sativa* after dipping in different concentration levels of each extract. After dipping for three minutes, the treated leaves were allowed to dry before offering to the nymphs. A day after treatment, all nymphs (treated and control) were provided with untreated food plant. Ten replicates (one nymph/replicate) were used for each concentration. Each individual nymph was isolated in a glass vial provided with a thin layer of sterilized sand as a floor.

III) Determination of Lactate Dehydrogenase Activity:

For the determination of the lactate dehydrogenase (LDH) activity in the fat body, samples of this tissue were collected from 5th instar nymphs of different ages (early, mid and late) and early adults, after treatment the early 4th instar nymphs. The fat body samples were weighed and then homogenized in a saline solution (the fat body of one insect / 1 ml saline solution 0.7 %) using a fine electric homogenizer, tissue grinder for 2 min. Homogenates were centrifuged at 4000 r.p.m. for 15 min. The supernatant was used directly or frozen until the use for the enzymatic determination. Three replicates were used and the fat bodies from two individuals were avoided to be mixed.

For the determination of the LDH activity in the haemolymph was collected from 5th (last) instar nymphs of the same ages and early emerged adults, after treatment the early 4th (penultimate) instar nymphs. Haemolymph was drawn into Eppendorff Pipetman containing few milligrams of phenoloxidase inhibitor (phenylthiourea) to prevent tanning or darkening and then diluted 5× with saline solution 0.7%. For whole blood assays, the diluted haemolymph was frozen for 20s to rupture the haemocytes. The haemolymph samples were then centrifuged at 2000 r.p.m. for 5 min, and only the supernatant fractions were used for assay directly or frozen until use. Three replicates were used and the haemolymph of two individuals were never mixed.

LDH activity was determined according to the method of (Tietz, 1999) using a kit of Biolap reagents. The enzyme was measured at wave length 340 nm by spectrophotometer.

IV) Statistical Analysis of Data:

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction (Moroney, 1956) for the test significance of difference between means.

RESULTS

1) LDH Activity in the Haemolymph of Nymphs and Adults of *S. gregaria*:

For investigating the effects of *F. bruguieri* extracts on the lactate dehydrogenase (LDH) activity in last instar nymphs and newly emerged adults, the penultimate instar nymphs were allowed to feed on clover leaves treated with each of two concentration levels of *F. bruguieri* extracted by methanol , petroleum ether or n-butanol. Table (1) contains data of affected LDH activity in the haemolymph of nymphs and adults. Unexceptionally, all *F. bruguieri* extracts induced the enzyme activity along the nymphal instar, irrespective of the solvent or concentration level but

significantly reduced it in adults. In the haemolymph of last instar nymphs, the least potent promoting effect on LDH activity was early exhibited (Change %: +0.5 in the early-aged nymphs at the lower concentration level of petroleum ether extract, Fig. 1 A) but the strongest enhancing effect was exhibited in the mid-aged nymphs (62601.3±467.4 U/L, in comparison with 53696.8±467.4 U/L of control congeners) after treatment with higher concentration level of methanolic extract.

Table (1): Effects of Fagonia bruguieri extracts on the lactate dehydrogenase activity (U/L) in haemolymph of the desert locust Schistocerca gregaria.

Solvent	Conc. %		Last instar nymphs			Newly emerged adults
			Early-aged	Mid-aged	Late-aged	
Methanol	7.5	Mean ± SD	59903.0 ± 809.5 c	62601.3 ± 467.4 d	65299.7 ± 934.7 d	60982.3 ± 467.4 b
	3.7	Mean ± SD	57204.7 ± 534.7 c	60712.5 ± 809.5 d	62601.3 ± 467.4 d	61791.8 ± 618.3 a
Petrolium ether	30	Mean ± SD	54776.2 ± 934.7 a	56665.0 ± 809.5 c	59633.2 ± 934.7 b	59633.2 ± 467.4 c
	15	Mean ± SD	54506.3 ± 467.4 a	56665.0 ± 809.5 c	58284.0 ± 809.5 a	60172.8 ± 467.4 b
n-butanol	30	Mean ± SD	55855.5 ± 809.5 a	54506.3 ± 467.4 a	60442.7 ± 934.7 b	57744.3 ± 467.4 c
	15	Mean ± SD	55046.0 ± 809.5 a	57204.7 ± 934.7 c	58553.8 ± 467.4 a	59633.2 ± 634.7 c
Controls		Mean ± SD	54236.5 ± 809.5	53696.8 ± 467.4	57474.5 ± 809.5	62871.2 ± 934.7

Conc.: Concentration, mean ± SD followed with the same letter (a): is not significantly different (P>0.05), (b): significantly different (P<0.05), (c): highly significantly different (P<0.01), (d): very highly significantly different (P<0.001).

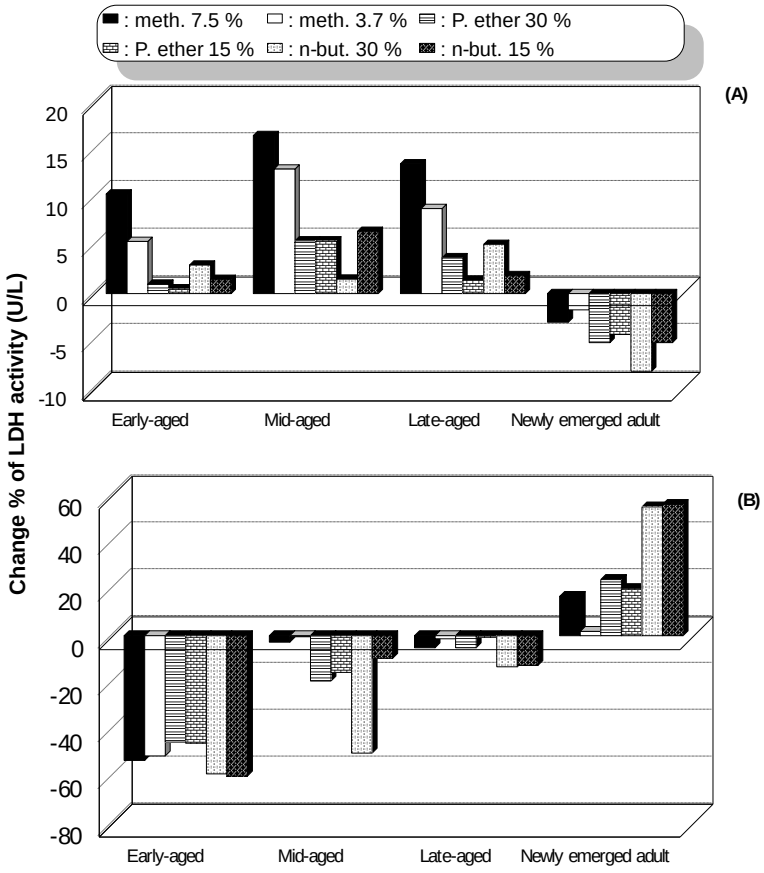

Fig. (1): Change % of lactate dehydrogenase activity in the haemolymph (A) and fat body (B) in the last instar nymphs of Shestocerca gregaria after treatment of the early penultimate instar nymphs with Fagonia brugueiri extracts (methanol, petroleum ether and n-butanol).

With regard to the newly emerged adults, the most drastically inhibited LDH activity in haemolymph was determined in 57744.3±467.4 U/L, after nymphal treatment with higher concentration level of n-butanolic extract (compared to 62871.2±934.7 U/L of control congeners). In addition, the least reducing effect of *F. bruguieri* was estimated after treatment with lower concentration level of methanolic extract (Change %: - 1.7, Fig. 1 A).

2) LDH Activity in the Fat Body of Nymphs and Adults of *S. gregaria*:

Just a look at data of Table (2) clearly shows the inhibitory effect of *F. bruguieri* extracts on the LDH activity in fat bodies of nymphs which reversed to be enhancing for such enzyme activity in the adults. All methanolic, petroleum ether and n-butanolic extracts of *F. bruguieri* prohibited LDH activity along the nymphs instar, irrespective of the concentration level.

Table (2): Effects of Fagonia bruguieri extracts on the lactate dehydrogenase activity (U/L) in fat body of the desert locust Schistocerca gregaria.

Solvent	Conc. %		Last instar nymphs			Newly emerged adults
			Early-aged	Mid-aged	Late-aged	
Methanol	7.5	Mean ± SD	10685.4 ± 161.9 d	11387.0 ± 93.5 a	8041.0 ± 186.9 b	11387.0 ± 93.5 d
	3.7	Mean ± SD	11117.7 ± 186.9 d	11656.8 ± 161.9 a	8364.8 ± 93.5 a	9929.9 ± 93.5 a
Petrolium ether	30	Mean ± SD	12466.3 ± 161.9 d	9444.2 ± 93.5 d	8041.0 ± 193.5 b	12088.5 ± 93.5 d
	15	Mean ± SD	12358.4 ± 93.5 d	9875.9 ± 161.9 d	8426.7 ± 186.9 a	11710.8 ± 93.5 d
n-butanol	30	Mean ± SD	9366.6 ± 107.4 d	5841.8 ± 83.5 d	7353 ± 58.4 d	15110.7 ± 116.8 d
	15	Mean ± SD	9166.4 ± 119.0 d	10583.8 ± 90.8 d	7399.2 ± 58.8 d	15215.6 ± 259.6 d
Controls		Mean ± SD	22839.5 ± 289.1	11710.8 ± 186.9	8475.0 ± 131.7	9750.3 ± 78.5

Conc., a, b, d: see footnote of Table (1).

The strongest reducing effect was early exerted on LDH activity in 9166.4±119.0 U/L (vs. 22839.5±289.1 U/L of control congeners) after treatment with the lower concentration level of n-butanolic extract and the least reducing effect was lately exerted as estimated in 11656.8±161.9 U/L after treatment with the lower concentration level of methanolic extract (compared to 11710.8±186.9 U/L of control congeners).

Opposing to the effect of *F. bruguieri* extracts on LDH activity in fat bodies of nymphs, these plant extracts pronouncedly enhanced the enzyme activity in the newly emerged adults. The most potent stimulatory effect on this enzyme activity was determined after treatment of the penultimate instar nymphs with lower concentration level of n-butanolic extract of *F. bruguieri* (Change %: +56.1, Fig. 1 B).

DISCUSSION

Dehydrogenases are very important tools for the investigation of insect metabolic activities during the course of development. The relative activities of the insect dehydrogenases may be related to the function and energy yielding demands of the tissues (Dickinson and Sullivan, 1975). Lactate dehydrogenase (LDH) (EC 1.1.1.28) is an important glycolytic enzyme that is present in virtually all tissues (Kaplan and Pesce, 1996). It is involved in carbohydrate metabolism and has been used as an indicative criterion of exposure to chemical stress (Diamantino *et al.*, 2001). LDH is, also, a parameter widely used in toxicology and in clinical chemistry to diagnose cell, tissue, and organ damage. However, the potential of this enzyme as an indicative criterion in the invertebrate toxicity tests has been scarcely explored (Ribeiro *et al.*,

1999; Senthil Nathan *et al.*, 2006b), in addition to its role as an evidence for an alternative pathway of terminal anaerobic metabolism (Bianconcini *et al.*, 1980).

1) *Disturbed LDH Activity in the Haemolymph:*

Unexceptionally, all *F. bruguieri* extracts induced the LDH activity in the haemolymph of the desert locust *S. gregaria*, in the present study, along the nymphal instar, irrespective of the solvent or concentration level. The strongest inducing effect was exhibited in the mid-aged nymphs (62601.3±467.4 U/L in comparison with 53696.8±467.4 U/L of control congeners) after treatment with the higher concentration level of methanolic extract. On the contrary, the LDH activity was significantly prohibited in the newly emerged adults. The most drastically inhibited enzyme activity (57744.3±467.4 U/L, compared to 62871.2±934.7 U/L of control congeners) was determined after nymphal treatment with higher concentration level of n-butanolic extract.

In general, the promoting effects of *F. bruguieri* extracts on LDH activity in haemolymph of nymphs of *S. gregaria* agree, to some extent, with those results reported for *Tribolium castaneum* by the insecticides permethrin and cypermethrin and not by malathion or pyrethroid (Saleem and Shakoori, 1987) and for the resistant strain of *Culex fatigans* by the insecticides DDT, malathion and cyfluthrin (Azmi *et al.*, 2002). Also, Abdel-Ghaffar and Basiouny (2007) estimated remarkably enhanced LDH activity in the haemolymph of last instar larvae of *Spodoptera littoralis* at 24 h post treatment with 30 microgram/larva precocene I as well as at 72 h post treatment with 150 microgram/larva.

Since LDH is important enzyme in the carbohydrate metabolism and related to energy production in the living cell, the induced activity level in the haemolymph of nymphs of *S. gregaria*, in the present study, indicates generally an active energy metabolism in this important tissue. It, also, may indicate an effective stimulation of the portion of the Cori cycle responsible for the overall recycling lactate, since this would result in concomitant enhanced production of pyruvate and glucose via glucogenesis (Harper *et al*., 1984).

2) *Disturbed LDH Activity in the Fat Body:*

Fat body is essential tissue for synthesizing certain metabolites and detoxification as well as storage in insects, hence the present study comprised the assessment of *F. bruguieri* extracts on LDH activity in this tissue. All *F. bruguieri* extracts prohibited LDH activity in the fat body along the last nymphs instar, irrespective of the concentration level. In contrast, the enzyme activity was pronouncedly enhanced in the fat body of adults, regardless to the extract. To a great extent, the present inhibitory effects of the *F. bruguieri* extracts are in agreement with those inhibitory effects of some insecticides and insect growth regulators (IGRs) on the LDH activity in fat bodies of other insects species such as the house fly *Musca domestica* (Hassanein *et al.*, 1996), the silk worm *Bombyx mori* (Nath, 2000), a susceptible strain of the mosquito *C. fatigans* (Azmi *et al.*, 2002), the rice leaffolder *Cnaphalocrocis medinalis* (Senthil Nathan *et al.*, 2006a, 2006b), the cotton leafworm *S. littoralis* (Abdel-Ghaffar and Basiouny, 2007). Investigating the inhibitory effect of certain *A. indica* extracts on LDH in another tissue, mid-gut, of *C. medinalis*, Senthil Nathan *et al.* (2006a) observed a decrease in the enzyme activity denoting a reduced metabolism in the insect and may be due to the toxic effects of neem derivatives on membrane permeability, especially on the gut epithelium (Senthil Nathan *et al.*, 2004, 2005 ; Smirle *et al.*, 1996).

The pyruvate is the key intermediate of glycolysis, whereas lactate is the end product of glycolysis. The interconvertability of pyruvate and lactate has a great

advantage in the operation of carbohydrate metabolism (Nath, 2000). The inhibited LDH activity in the fat body of *S. gregaria* by *F. bruguieri* extracts, in the present study, indicate the inhibition of lactate conversion to pyruvate, resulting in a shift from aerobic to anaerobic metabolism for meeting the required energy demands under toxic conditions.

It is clear from our results that the normal physiological functioning of haemolymph and fat body of *S. gregaria* is greatly disturbed after treatment with *F. bruguieri* extracts. The disturbed LDH activities in haemolymph and fat body indicate that the toxic components contained in the present plant extracts might be affecting the synthesis or functional levels of LDH, directly or indirectly, by altering the cytomorphology of the cells (Nath, 2000). In addition, the increase and decrease, or induction and inhibition, of the LDH activities, as recorded in the present study on *S. gregaria*, might be, on molecular levels, referred to depression or mutations of the regulating genes responsible for biosynthesis of polypeptide chains building this enzyme (Hassanein *et al.*, 1996). However, further investigations should be needed to clarify the precise role of these extracts from the wild plant *F. bruguieri* in the gene promotion, rate of expression and eventually synthesis of nucleic acid and enzymatic peptides in the target tissues.

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ARABIC SUMMARY

التغيرات الحاصلة في نشاط إنزيم لاكتيت ديهيدروجينيز بفعل مستخلصات النبات البري *فاجونيا بروجيري* (الفصيلة الطرطراوية) في الجراد الصحراوي *شيستوسركا جريجاريا*

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تمّ بحث تأثير مستخلصات النبات البري المسمى "حُلاوى" (*فاجونيا بروجيري*) في أنشطة إنزيم لاكتيت ديهيدروجينيز (ل هـ) في الهيموليمف والأجسام الدهنية لحوريات الدور الأخير واليافعات حديثة البزوغ للجراد الصحراوي *شيستوسركا جريجاريا* . ومن أجل ذلك، أُعطيت حوريات الدور قبل الأخير واحدا من تركيزين من المستخلصات التي استخدمت لها المذيبات : ميثانول (7,5 ، 3,7%) ، إثير بترولي (30,0، 15,0%)، ن- بيوتانول (30,0، 15,0%)، وذلك عن طريق معاملة الغذاء الطازج الذي تناولته ، وهو نبات البرسيم.

سجلت نتائج البحث الحالي تأثيرا تحفيزيا لمستخلصات *فاجونيا بروجيري* في نشاط الإنزيم (ل هـ) الموجود في هيموليمف الحوريات، على امتداد عمرها ، بلا استثناء، بغض النظر عن نوع المذيب المستخدم أو التركيز المستعمل، ولكنه كان تأثيرا تثبيطيا في اليافعات حديثة البزوغ . وقد ظهر أقوى تأثير تحفيزي في الحوريات متوسطة العمر (62601,3±467,4 وحدة/ليتر) بعد المعاملة بالتركيز الأعلى من المستخلص الميثانولي (مقابلة بالقيمة 53696,8±467,4 يو/ل المسجلة للحوريات الضابطة). وعلى النقيض، فقد أدى النبات المختبر إلى تثبيط نشاط الإنزيم تثبيطا ملحوظا في هيموليمف اليافعات حديثة البزوغ، وكان أقوى تأثير مثبّط لنشاط هذا الإنزيم في الهيموليمف مقدرا بالقيمة 57744,3±467,4 وحدة/ليتر بعد معاملة الحوريات بأعلى تركيز من المستخلص البيوتانولي (مقابلة بالقيمة 62871,2 ±934,7 وحدة/ليتر المسجلة لليافعات الضابطة).

وبالنسبة لنشاط هذا الإنزيم في الجسم الدهني ، فلقد تم تثبيطه على امتداد الدور الحوري، بغض النظر عن نوع الـمذيب المستخدم أو التركيز المستعمل من المستخلص به . وكان أشد اختزال لنشاطه مقدرا بالقيمة 9166,4±119,0 وحدة/ليتر (مقابلة بالقيمة 22839,5±289,0 وحدة/ليتر للحوريات الضابطة) بعد المعاملة بالتركيز المنخفض من المستخلص البيوتانولي . وعلى النقيض، فقد شجّعت المستخلصات النباتية الحالية نشاط هذا الإنزيم في الجسم الدهني الموجودة في اليافعات، بصرف النظر عن نوع المذيب المستخدم . وظهر أقوى تأثير تحفيزي في اليافعات بعد معاملة الحوريات بأقل تركيز من المستخلص البيوتانولي.