



## Change in malate dehydrogenase and alpha amylase activities in *Rubus fruticosus* and *Valeriana jatamansi* treated granary weevil, *Sitophilus granarius*

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Received: August 16, 2019 – Accepted: November 27, 2019 – Distributed: May 31, 2021

(With 1 figure)

### Abstract

Poor storage conditions provide favorable environment to stored grain pests for their growth. The bio-pesticides are the best alternatives to synthetic pesticides. Present study was conducted to compare toxicity of *Rubus fruticosus* and *Valeriana jatamansi* against granary weevil, *Sitophilus granarius* and subsequent changes in enzyme activity responsible for grain damage. In current research 5 g of *R. fruticosus* fruit and *V. jatamansi* rhizome powders were tested separately against *S. granarius*, in 50 g wheat whole grains for seven days in comparison with the control. The enzymatic activity of malate dehydrogenase and  $\alpha$ -amylase was observed in the cellular extracts of *S. granarius*. The insects were crushed and homogenized in phosphate-buffer solution and centrifuged at 10000 rpm for 5 minutes. For the enzymatic measurement supernatant was tested; the spectrophotometer was adjusted at 340 nm. The reagents were mixed and incubated at 25 °C for five minutes. The cuvettes were placed in the experimental and reference sites of spectrophotometer and recorded the change in absorbance for 3-4 minutes. There was 5.60% and 14.92% reduction in the activity of malate dehydrogenase in *R. fruticosus* and *V. jatamansi*, treated insects, respectively. The alpha amylase enzyme activity was 6.82% reduced and 63.63% increase in *R. fruticosus* and *V. jatamansi*, treated insects, respectively. Present study addresses that both plant powders are effective against granary weevil by altering enzyme activities so both the plant powders can be used as bio-pesticides against the stored grains pests.

**Keywords:** granary weevil, pests, biopesticides, malate dehydrogenase,  $\alpha$ -amylase.

## Mudança nas atividades de malato desidrogenase e alfa amilase em *Rubus fruticosus* e gorgulho tratado com *Valeriana jatamansi*, *Sitophilus granarius*

### Resumo

As más condições de armazenamento proporcionam um ambiente favorável às pragas armazenadas para o crescimento. Os biopesticidas são as melhores alternativas aos pesticidas sintéticos. O presente estudo foi conduzido para comparar a toxicidade de *Rubus fruticosus* e *Valeriana jatamansi* contra gorgulhos, *Sitophilus granarius* e subsequentes alterações na atividade enzimática responsáveis por danos aos grãos. Na pesquisa atual, 5 g de frutos de *R. fruticosus* e pós de rizoma de *V. jatamansi* foram testados separadamente contra *S. granarius*, em 50 g de grãos integrais de trigo por sete dias, em comparação com o controle. A atividade enzimática da malato desidrogenase e  $\alpha$ -amilase foi observada nos extratos celulares de *S. granarius*. Os insetos foram esmagados e homogeneizados em solução tampão fosfato e centrifugados a 10000 rpm por 5 minutos. Para a medição enzimática, o sobrenadante foi testado; o espectrofotômetro foi ajustado a 340 nm. Os reagentes foram misturados e incubados a 25 °C por cinco minutos. As cubetas foram colocadas nos locais experimentais e de referência do espectrofotômetro e registradas as alterações na absorbância por 3-4 minutos. Houve redução de 5,60% e 14,92% na atividade da malato desidrogenase em *R. fruticosus* e *V. jatamansi*, insetos tratados, respectivamente. A atividade da enzima alfa amilase foi reduzida em 6,82% e aumento de 63,63% em *R. fruticosus* e *V. jatamansi*, insetos tratados, respectivamente. O presente estudo aborda que ambos os pós de plantas são eficazes contra o gorgulho do celeiro, alterando as atividades enzimáticas, de modo que ambos os pós de plantas possam ser usados como biopesticidas contra pragas de grãos armazenados.

**Palavras-chave:** gorgulho, pragas, biopesticidas, malato desidrogenase,  $\alpha$ -amilase.

## 1. Introduction

Cereals are main source of human diet with production exceeding 2100 million tons annually (Shewry, 2007). Bulk of harvested crop (50%) is lost during storage (Fornal et al., 2007). The poor storage conditions provide favorable environment for the growth of stored grain pests (Stoll, 2000). Insect pests of cereals are responsible for reduction in quantity and quality of food grains (Udo, 2011). The weight losses in stored products are mainly associated with activities of insect pests (Arlian, 2002). According to an estimate, annual yield losses due to insect pest are 20-40 percent in overall agricultural products (FAO, 2018). About 1000 insect pests have been reported in harvested products worldwide (Atwal and Dhaliwal, 2008). Many strategies have been adopted to preserve cereals from pest infestations; including pesticides, bio pesticides, repellants, fumigants and biological control measures (Germinara et al., 2008).

Synthetic insecticides are widely used against insect pests (Tapondjou et al., 2005). These pesticides cause environmental pollution and develop resistant insect pests and high mammalian toxicity (Al-Moajel, 2000). Development of bio-insecticides has been an alternative strategy to use of synthetic pesticides (Hashim and Devi, 2003; Meena et al., 2006). In Asia a number of plant products have been traditionally used for protection of stored cereals (Isman, 2000). Plants derivative pesticides are renewable, biodegradable and have low mammalian

cytotoxicity (Isman, 2008). Digestive enzymes convert complex biomolecules to simplest *one* (Erturk, 2006). Any disturbance in enzyme activity of insects is fatal for their survival (Shekari et al., 2008). Dehydrogenases are important tools for the investigation of insect metabolic activities during the course of development (Dickinson and Sullivan, 1975). Terra and Ferreira (2005) confirmed that Amylases are obligatory to digest carbohydrates in insects.

## 2. Material and Methods

### 2.1. Sampling area

The infested wheat grains with *S. granarius* were randomly collected from wheat stores of Mansehra and reared in the laboratory. Local wheat, *Triticum aestivum* was used for rearing of insect. The wheat used in the experiment was cleaned from insects by fumigation with Aluminum sulphate. Fresh, mature and unripen fruits of *R. fruticosus* were collected from Shatey, Mansehra. The rhizomes of *V. jatamansi* were collected from Kund Bangla, Mansehra, Pakistan (Figure 1).

### 2.2. Rearing of insects

Three hundred adult *S. granarius* were grown in 2-liter jar (14×13.6 cm) having 750 g purified wheat grains at a controlled temperature of  $37 \pm 2$  °C and relative-humidity  $60 \pm 10\%$  (Mehmood, 2007). Each rearing jar was covered with muslin cloth for aeration.



**Figure 1.** (a) Plant of *V. jatamansi*; (b) dried rhizome of *V. jatamansi*; (c) Plant of *R. fruticosus*; (d) dried fruits of *R. fruticosus*.

### 2.3. Preparation of plant powders

Mature and unripened fruits of *R. fruticosus* and rhizome of *V. jatamansi* were collected, rinsed with distilled water and air dried in the laboratory at room temperature of 20 °C. Dried plant materials were grounded by electrical blender (Rehman et al., 2009). The resulting powders were sieved, using 40 mesh screens and stored in cool and dry place to maintain efficacy.

### 2.4. Determination of malate dehydrogenase activity

The enzymatic activity of malate dehydrogenase (MDH) was observed in the cellular extract of *S. granarius* after treatment with *R. fruticosus* and *V. jatamansi* powders and compared with control. The experimental insects were crushed and homogenized in phosphate buffer (pH 7.0) solution using a mortar and pestle. Homogenates were centrifuged (Force 1624 microcentrifuge, Edison, NJ, USA) at 10000 rpm for 5 minutes. For the enzymatic measurement of malate dehydrogenase, supernatant was tested in spectrophotometer adjusted at 340 nm. The following solutions were added into the cuvette (1.4 mL Phosphate buffer (0.1M), 0.04 mL Cis-Oxaloacetic acid (0.006M) and 0.02 mL NADH (0.00375M). The reagents were mixed and incubated at 25 °C for five minutes. The cuvettes were placed in the experimental reference sites of spectrophotometer (Perkin Elmer lambda 25 UV/Visible, double-beam, Spectrophotometer, USA) for 2-3 minutes to achieve temperature equilibration, and pipetted 0.005 mL extract of insects into experimental cuvette, mixed it well and monitored the reaction for 2-3 minutes. The oxaloacetate and NADH is converted into L-Malate and NAD<sup>+</sup> by the enzymatic action of Malate dehydrogenase:

#### MDH



The measurement of MDH activity was based on the rate of decline in the absorbance at wave length ( $\lambda$ ) of 340 nm, subsequent to the oxidation of NADH. One enzyme unit is defined as the amount of enzyme which can catalyze the conversion of one  $\mu$ mole of oxaloacetate to malate per minute at 25 °C. The number of enzyme units per mL of cellular extract was calculated by using the Formula 1:

$$\text{Enzyme activity (U / mL)} = \frac{\text{Absorbance change} \times \text{Total volume (mL)} \times \text{DF}}{6.22 \times \text{Enzyme volume (mL)}} \quad (1)$$

Whereas: DF = Dilution factor of enzyme; 6.22 = Extinction coefficient of NADH at 340 nm in milli moles (Worthington and Worthington, 2011).

### 2.5. Determination of alpha amylase activity

Enzymes dilutions were prepared using Sodium Phosphate ( $\text{Na}_3\text{PO}_4$ ) 0.02 M buffers at 6.9 pH for each strain separately in eppendorf tubes and kept in ice box. One gram of dinitrosalicylic acid as color reagent was mixed in 20 mL of 2M sodium hydroxide (NaOH) and slowly added 30 g sodium potassium tartrate tetrahydrate and made final volume to 100 mL. One percent starch was dissolved in Sodium phosphate buffer (0.02M) having 0.006M sodium chloride (NaCl). 500  $\mu$ L of 1% starch solution was mixed with 20  $\mu$ L of cellular extract in triplicates. One ml of the salicylic acid solution was added to each reaction mixture and boiled for five minutes. The reacted samples were cooled to room temperature and added 10  $\mu$ L of distilled to each tube (making 6.66 time dilution). The absorbance of diluted reaction mixture was measured at 540 nm and compared with standard curve of maltose for the determination of  $\mu$ -moles of maltose liberated in the reaction. A unit of amylase is defined as the amount of enzyme which can liberate one  $\mu$ -moles of maltose from starch in one minute (Worthington and Worthington, 2011). The activity of enzyme was calculated as follows (Formula 2):

$$\text{Enzyme activity (U / mL)} = \frac{\mu \text{ moles of maltose liberated per minute}}{\text{Volume of enzyme added (mL)}} \quad (2)$$

## 3. Results

The reduction in malate dehydrogenase activity for *R. fruticosus* and *V. jatamansi* against *S. granarius* revealed a decrease in the levels of enzyme by 5.60% and 14.92% respectively, compared with that of control (Table 1).

The activity level of amylase in the extract of *R. fruticosus* and *V. jatamansi* treated insects showed 6.82% decrease and 63.63% increase respectively. The results represented a significant decrease in the  $\alpha$ -amylase activity in *V. jatamansi* treated insects while a modified activity was observed in *R. fruticosus* treated *S. granarius* (Table 2).

**Table 1.** Reduction in malate dehydrogenase activity for *R. fruticosus* and *V. jatamansi* against *S. granarius*.

Treatments (g)	Units of Enzyme (MDH)*	%	Change in enzyme activity
Control	9.1274	100	—
<i>R. fruticosus</i>	8.6166	94.40	5.60% decrease
<i>V. jatamansi</i>	7.7648	85.08	14.92% decrease

\*Unit of enzyme: amount of enzyme which can catalyze the conversion of one  $\mu$ mole of oxaloacetate to malate per minute at 25 °C.

**Table 2.** Alteration in alpha amylase activity for *R. fruticosus* and *V. jatamansi* against *S. granarius*.

Treatments	Units of enzyme (amylase)*	%*	Change in enzyme activity
Control	7359	100	—
<i>R. fruticosus</i>	6857	93.18	6.82% decrease
<i>V. jatamansi</i>	12042	36.37	63.63% increase

\*One unit enzyme: amount of enzyme which can liberate one  $\mu$ mole of maltose from starch in one minute.

#### 4. Discussion

This study was carried out from May-September, 2011 to assess the efficacy of two plant powders *R. fruticosus* and *V.jatamansi* against *S. granarius* and changes in the enzyme activities. Both the plant powders were collected from different localities of Mansehra Pakistan and were used as treatment against the granary weevil.

In the present study the malate dehydrogenase enzyme activity in *R. fruticosus* and *V. jatamansi* treated *S. granarius* was reduced by 5.60% and 14.92% respectively, compared with that of control. In the previous studies of Hamadah et al. (2010) the alterations in the dehydrogenase activity of the desert locust *Schistocerca gregaria* was observed, the wild plant *Fagonia bruguieri* changed the enzyme activity +0.5% in the early-aged nymphs at the lower concentration level of petroleum ether extract along the nymphal instar but the strongest enhancing effect was exhibited in the mid-aged nymphs.

In the present study the  $\alpha$ -amylase enzyme activity in *R. fruticosus* and *V. jatamansi* treated *S. granarius* 6.82% decrease and 63.63% increase respectively. Earlier Nehad et al. (2008) reported that activity of amylase was decreased after 24h of plant extract treatment. Mehrabadi et al. (2011) reported Inhibitory effect of plants extracts of *A. Siberia*, *P. harmala*, and *T. vulgaris* against *C. maculatus* showed the reduced amylase activity 19.22%, 4.58%, and 7.22% respectively. Ahmad et al. (2019) used different plant powders against *T. castaneum* infesting stored grains. *Allium sativum* and *Zingiber officinale* were more effective resulting into 15 time's higher adult mortality. The *Azadirachta indica* seed powder against the beetle showed better control at lowest (1% w/w) and the highest doses (5% w/w).

#### Acknowledgements

Dr. Muhammad Shahid Nadeem is appreciated to accomplish my enzyme assay on the bio-pesticides, treated insects.

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