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House fly control using natural products as amylase inhibitors

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Abstract
House flies are vectors for many pathogens and hence medically important pest too. The control of flies is a challenge. The use of chemical insecticides is this regards though effective has not solved the problem. The intensive use of broad-spectrum insecticides against *M, domestica* has led to the development of resistance to many registered pesticides use for its control. Thus it is necessary to find a new safe and cheap alternative to manage fly populations. Plant products as an alternative to conventional chemical method have now been established worldwide. The purpose of the present study is to find out a plant based inhibitor for the digestive enzymes of this pest. The physiology and biochemistry of the insect digestive enzyme has an important role in the study of novel insecticidal strategies. Thus in this study two plant extracts have been assessed for their α-amylase inhibitory activity of house adult and larvae.

Keywords: *Musca domestica* L. insecticides, amylase, inhibition, *Lantana camera*, *Eucalyptus grandis*

Introduction

The common housefly *Musca domestica* L. (Diptera: Muscidae) is a well-known cosmopolitan pest of both farm and home that possesses a serious health threat to human beings and livestock by transmitting many infectious diseases like dysentery, cholera, diarrhea and typhoid because it acts as important mechanical carriers of several pathogenic bacteria such as Shigella dysenteries, Vibrio cholerae, Escherichia coli and Salmonella typhi. (Greenberg, B. 1973) [15].

To control the of house fly population, chemical insecticides are commonly used. But resistance has been developed against insecticides used for its control. Additionally, these flies are well-known for their capability to develop metabolic and behavioural adaptations to abstain and for the detoxification of synthetic insecticides (Attaullah Muhammad Kashif Zahoor *et al* 2019) [1]. The huge costs of chemical insecticides and concerned environmental harmful impacts encouraged scientists to search less toxic, eco-friendly and cheaper biopesticides as alternative way for the pest management in future (Sultana *et al*., 2016). Plant-derived products have emerged as a leading source of alternatives for pest control. Plant extracts have bioactive compounds (polyphenols, alkaloids, terpenoids, essential oils, steroids, lignin, fatty acids, and sugars) which are basically secondary metabolites of plants and show broad spectrum of actions against variety of insect pests (Attaullah Muhammad Kashif Zahoor, *et al* 2019) [1]. The advantages of botanical insecticides are mainly due to their quick degradation and lack of persistence and bioaccumulation in the eco system, which have been key problems in the use of chemical insecticides. Several experiment with botanical insecticides, shows they are degraded in the environment in hours or days. Further literature has clearly shown that use of plant natural products provides low risk when compare with chemical insecticides. The availability and diversity of the secondary metabolites in plant extracts is renewable Source. Multiple analogs of one plant origin compound, is known to increase the efficiency through synergism, reduce the rate of metabolism of the compounds and prevent the pest resurgence/pesticide resistance (Senthil-Nathan *et al* 2004, 2005, Ntalli and Menkissoglu-Spiroudi, 2011) [5]. Therefore, these products are considered as good substitutes to chemical insecticides for insect pest control and have little effect on human health, environment and predators, parasites and pollinator insects due to minimum residual activity (Regnault-Roger *et al*., 2012) [2].

The α-amylase (α-1, 4-glucan-4-glucanohydrolases; EC 3.2.1.1) is a hydrolytic enzyme, found in microorganisms, plants and animals. This enzyme is most important for the digestion of starch and related carbohydrates (Strobl *et al*., 1998). Starch digestion by insect amylases has been demonstrated and described in several insect species, including, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) (Darvishzadeh *et al*., 2012) [12], *Ceratitis capitata* (Wiedemann)(Diptera: Tephritidae) (Darvishzadeh *et al*., 2013) [12] *Sitophilus oryzae* Hustache (Coleoptera: Curculionidae) (Baker and Woo, 1985) [6], and *Zabrotes subfasciatus* (Bohemann) (Coleoptera: Bruchidae) *Macrosiphum rosae* (Hemiptera: Aphididae) (Darvishzadeh and Bandani, 2012) [11] and *Tecia solanivora* Povolny larvae (Lepidoptera: Gelechiidae) (Valencia-Jimé-nez *et al*., 2000) [25].

The physiology and biochemistry of the insect midgut has an important role in the study of novel insecticidal strategies. Digestive enzymes could be aimed by plant inhibitors to interfere in food digestion and its absorption in pests (Jongsma and Bolter, 1997; Gatehouse and Gatehouse, 1999). Inhibitors of insect digestive enzymes have important role in the control of insect pests. Dias *et al*. (2010) [13] demonstrated that rye α-amylase inhibitor expressed in transgenic tobacco seeds (*Nicotiana tabacum*) caused 74% mortality in *Anthonomus grandis*, first larval instar when transgenic seed flour mixture used in artificial diet.

Understanding the biochemistry and physiology of nutrition and feeding adaptation is important in inhibitors.

Most insects depend on their amylases and other enzymes for efficient digestion of their food. For both the Maintenance of adult longevity and optimal larval growth, carbohydrates are essential for initial hydrolyzing of energy-producing nutrients (Dadd, 1985) [10]. The present work deals with the study of α-amylase inhibitor activity of two plant extracts viz. *Lantana camara* Linn. And *Eucalyptus grandis*.

Lantana camara Linn. (Fig 1.a) is a flowering ornamental plant belonging to family Verbenaceae. *L. camara* is also known as Lantana, Wild Sage, Surinam Tea Plant, Spanish flag and West Indian lantana. It is a well-known medicinal plant in traditional medicinal system.

It *is* a low erect or subscandent vigorous shrub with tetrangular stem, stout recurved pickles and a strong odour of black currents. Plant grows up to 1 to 3 meters and it can spread to 2.5 meter in width. Leaves are ovate or ovate oblong, acute or sub-acute, crenate serrate, rugose above, scabrid on both sides. The leaves are 3-8 cm long by 3-6 cm wide and green in colour. Leaves and stem are covered with rough hairs. Small flower held in clusters. Colour usually orange, sometime varying from white to red in various shades and the flower usually change colours as they age (Khare, 2007; Kirtikar and Basu 2006; Chopra, *et al*. 1956) [18, 19, 9] Different parts of *L. camara* are reported to possess essential oils, phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids, glycosides, iridoid glycosides, phenyl ethanoid, oligosaccharides, quinine, saponins, steroids, triterpens, terpenoides and tannin as major phytochemical groups (Ganjewala, *et al* 2009; Kensa, 2011) [14]

Eucalyptus grandis (Fig 1.c) attains a height of 45-55 m, usually with an excellent trunk and a widespreading, rather thin crown; most of the bark and branches are smooth, white or silvery, sometimes greenish, rough on lower stem, smooth above, debark easily. Juvenile leaves are petiolate, opposite for several pairs then alternate, ovate up to 16 x 8.5 cm, green to dark green and slightly wavy; adult leaves are petiolate, alternate, stalked, lanceolate to broad lanceolate, up to15x3 cm, green on topside and pale green on underside, slightly wavy, with a long point. Inflorescence axillary and simple, 7 flowered; peduncules flattened, to 1.8 cm long; buds have a bluish bloom. Fruit or seed capsules several, short stalked, pear shaped or conical, slightly narrowed at the rim, thin, 8x6 mm, with whitish waxy coating, narrow sunken disc, and 4-6 pointed, thin teeth, slightly projecting and curved inward, persisting on twigs. (Orwa, *et al*, 2009) [20] The main constituents of the oil of the *E. grandis* are *α*-Pinene (29.69%), p-Cymene (19.89%), 1, 8- cineole (12.80%), *α*-Terpineol (6.48%), Borneol (3.48%) and 3.14% D-Limonene (Oluwagbemiga, *et al*. 2013) [21].

Materials and Methods

Plant materials -The plant material of *Lantana camara* Linn and *Eucalyptus grandis*, were collected from and around Pune, Maharashtra, India, The collected material was washed with distilled water and dried in shade at room temperature. The dried material was then powdered separately and subjected to soxhlet apparatus for60-70hrs. The extracts thus obtained were then concentrated in rotary evaporator. All extracts were dissolved in AR grade acetone, and serial dilutions were prepared as per requirement for the experimental purpose.

Rearing of housefly colony

The nucleus culture of *M. domestica* was obtained from Entomology Section, National Chemical Laboratory, Pune. The colony was maintained at 28±2 °C and 70–75 % relative humidity. Adults were reared in 30×30×30-cm metal frame cages covered on all sides with muslin cloth, cotton swab served as substrate for oviposition.

Insects and enzyme extraction

Enzyme samples from the alimentary tract (Foregut, Midgut and Hindgut) were prepared based in Darvishzadeh *et al* (2013) [12]. Briefly, larvae were placed on ice (about 5 min) for immobilization and dissected under binocular. After dissection, different parts of alimentary tract were removed and homogenized using a manual glass homogenizer. The homogenates were centrifuged at 15,000 ×g for 15 min at 4 ºC and the supernatants were kept at -20 ºC as the enzyme source. The adult flies were dissected and the guts obtained were treated as mentioned above.

A-Amylase activity

A-Amylase activity was assayed by the dinitrosalicilic acid (DNS) procedure (Bernfeld, 1955), using 1% soluble starch solution as substrate as described by Bandani *et al*. (2009) [7]. One unit of α-amylase activity was defined as the amount of enzyme required to produce 1 mg maltose in 30 min at 35ºC. A blank without substrate but with α-amylase extract and a control containing no α-amylase extract with substrate were run simultaneously with reaction mixture. All assays were performed in triplicates.

Results and Discussion

Most insects depend on their amylases and glucosidases for efficient digestion of their diet. For both the maintenance of adult longevity and optimal larval growth, carbohydrates are essential for initial hydrolyzing of energy-producing nutrients (Dadd, 1985) [10]. Interruption in carbohydrate metabolism can prove to be a way to keep the insect population under control. An attempt on the same line has been made to study the inhibitory action of the selected plant extracts on α-amylase of larval and adult house flies.

The enzyme fractions obtained were incubated with the respective plant extracts and the activity was measured. The results obtained are given in table 1 and table 2. The amylase activity without the incubation of the plant extracts (control) is given in table 1 whereas the influence of the extracts on the amylase activity is evident from table 2. Both the extracts have certainly inhibited the amylase activity but the inhibitory action of the *Lantana camera* is more as compared to *Eucalyptus grandis*. These plants can be included as potent agents in controlling house flies.

Table 1: α-Amylase activities of the gut of *M.domestica* larvae and adults (Control)

Enzyme	Organ	Total activity (µmol/min)	Total protein (mg)	Specific activity (µmol/min/mg protein)
α-amylase	Larval gut	0.71± 0.19	1.36	0.50 ± 0.05
	Adult gut	1.74 ± 0.27	2.42	0.70 ± 0.11

Table 2: α-Amylase, incubated with plant extracts, activities of the gut of *M. domestica* larvae and adults

Enzyme	Organ	Plant extract (1 %)	Total activity (µmol/min)	Total protein (mg)	Specific activity (µmol/min/mg protein)
α-amylase	Larval gut	*E. grandis*	0.49± 0.20	1.37	0.37 ± 0.19
		L. camera	0.36 ± 0.11	1.37	0.29 ± 0.23
	Adult gut	*E. grandis*	1.04 ± 0.16	2.45	0.43 ± 0.29
		L. camera	0.96 ± 0.29	2.47	0.41 ± 0.09

Insects generally use different types of digestive enzymes secreted by midgut epithelial cells. These enzymes allow them to digest a wide range of food diets including polymeric molecules. Some car bohy drases and proteases can break down the molecules into absorbable elements in midgut (Terra WR at al 1994, 1996; Reeck *et al* 1999) [23, 22]. The alteration of digestive enzymes provides some insights about the mode of action of the plant extracts. Through the present work is a new avenue of controlling flies by blocking their digestive enzymes is brought into light.

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