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ORIGINAL ARTICLE

Effect of different diets on *Tyrophagus putrescentiae* population and amelioration of their immunological disorder by garlic

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Abstract
Background: The storage mite, *Tyrophagus putrescentiae*, detected in the samples collected from stored products and house dust, is one of the major causes of allergic disorders.
Objective: The purpose of this study was to ameliorate the *T. putrescentiae* faeces allergic immunological disorder by garlic.
Methods: Albino experimental rats were classified into control, inhaled and treated groups. Mass rearing of *T. putrescentiae* on different diets, and ELISA of some cytokines and IgE techniques were used.
Results: The results obtained showed the highest population of *T. putrescentiae* reared in four from thirteen tested diets. In addition, significantly higher serum levels of INF-γ and IgE were found in rats treated with faeces than the other groups; especially the garlic-treated group. In contrast, IL-4 was lower in faeces-treated rats than the others; however, the control group had the highest level of IL-4. Statistical analysis of data showed a significant difference between the garlic-treated group and either control or faeces-treated groups ($P < 0.05$).
Conclusions: The population of *T. putrescentiae* mites peaked in four from thirteen tested diets. The immunological disorder caused by repeated exposure to *T. putrescentiae* faeces might be modulated by garlic.

Introduction

Medicinal plants have been used to cure human illness since ancient times. Certain types of these plants are believed to promote positive health and maintain organism resistance against infection by re-establishing body equilibrium and conditioning the body tissues. Among these plants, garlic (*Allium sativum*) a traditional dietary and medicinal applications as an anti-microbial agent[1] is a common food spice widely distributed and used in all parts of the world as a spice and herbal medicine for the prevention and treatment of a variety of diseases, ranging from infections to heart diseases.[2] Garlic is thought to have various pharmacological properties and medical applications. It is mainly consumed as a condiment in various prepared foods.[3] This prompted us to propose garlic as an asthmatic modulator medicinal plant.

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Understanding the nature of environmental triggers is fundamental in attempts to prevent/reduce allergic diseases. As previously noted, exposure to common aeroallergens, especially perennial inhalable allergens such as mite, is associated with a significantly increased risk for asthma.[4] Mite infestation is associated with negative effects on man and his resources. Storage mites consume stored grain and oil seeds, transfer toxicogenic microorganisms and produce allergens, thereby causing occupational allergies and endangering food safety.[5] The storage mite, *Tyrophagus putrescentiae* is found in stored products such as dried eggs, ham, herring meal, cheese, and different kinds of nuts.[6] Storage mites are the cause of occupational allergic diseases of bakers[7] and food industrial workers[8,9] and among residents in agricultural environments.[10] En-Chih et al. had also mentioned that both *T. putrescentiae* and *Dermatophagoides pteronyssinus* are causative factors for the development of airway hypersensitivity.[11] The mass rearing processes were also reported elsewhere.[12,13]

The storage mite *T. putrescentiae* causes allergic response and IgE production in animals.[14] The asthma symptoms are caused by inhalation of live mites and fragments of dead mites, or their excretory pellets.[15]

Bronchial asthma is a chronic inflammatory disease of the airway.[16] Chronic exposure to allergens triggers a distinct array of immunobiological and biochemical reactions that directly stimulate and induce abnormalities of air-way structure resulting in the development of clinical symptoms.[17] Allergic disorder symptoms are associated with high levels of serum allergen-specific IgE and eosinophilia.[18] In addition, both allergen exposure protocols result in immune-mediated airway inflammation defined by elevated levels of IgE, the T-helper cell 2 (TH2) cytokines IL-4 and eosinophils.[19] The pro-inflammatory cytokine, IFN-γ, promotes T-helper type-1 (Th1) responses, which down-regulate the Th2-like immune responses that are hallmarks of allergic diseases, including asthma.[20]

The objective of the present investigation was to develop mass rearing techniques of this species by 13 different natural foods and to examine their immunological disorders in male albino rats. Modulation of these disorders was tested by garlic.

Materials and methods

Mass rearing of *Tyrophagus putrescentiae*

The choice of the effective diets or feeding media is necessary for mass rearing of allergic mites, because the antigen preparation needs a very big numbers of allergic mites. Stored product samples, collected from El-Minia Governorate, were used for establishing a stock culture of *T. putrescentiae*. Live mites were isolated by a modified Berlese funnel with a wire screening[21] covered with muslin. Each sample was placed on the muslin and spread. Mites which escaped from light were received in a Petri dish. The bottom of the Petri dish is filled with a paste made of a mixture of charcoal and gypsum (2:8 parts) to construct a porous medium.[22] The rim of the dish is coated with a phaslene. Mites were sorted out, preserved and mounted on microscope slides for species identification.[23,24] *T. putrescentiae* figures were captured by light microscopy at 400× and 600× magnifications.

The following culture media obtained from local market were compared: (1) wheat germ, (2) roomy cheese, (3) nedo, (4) egg yolk, (5) potatoes, (6) fish meal, (7) copra, (8) egg albumin, (9) chicken bouillon, (10) pollens, (11) powdered bean, (12) human hair and scales, and (13) yeast extract.

Five males and five females in copulation state were caged with the tested diet in certain plastic vials (5 mm diam. × 20 mm length), cigarette paper held in place by a snap-on plastic ring confined the mites and allowed gas exchange between the culture environment and a humidity chamber environment. The bottom of each vial was filled with the last described paste. Each combination of food and species of mites was replicated five times within a test. The Petri dishes of the stock culture and the plastic vials of the tested diets were carried out at 25 ± 1 °C and 75% relative humidity (RH). The temperature was adjusted at 25 °C by placing the entire system inside an incubator. The relative humidity inside the desiccators was maintained at 75% by the concentration of the KOH (22.25 g of KOH per 100 ml dis. water). The concentration is also sufficient to absorb the CO_2 as soon as it is released.[25] Every two months the stock cultures were transplanted into fresh media to prevent outgrowth and a consequent breakdown of the population. Contamination with fungi was controlled by: (1) sterilising the tested diets and containers using dry heat (roughly 90 °C for 30 min). (2) Adding diets in small crumbled amounts. (3) Using of 75% RH instead of 80%, the deal RH needed for *T. putrescentiae* culture. The procedure of mite counts was as mentioned by Ree and Lee.[26]

Crude extracts

When the population density was sufficiently high, *T. putrescentiae* crude faeces were isolated by the Berlese–Tullgren method by photonegative live mites escaping. Five grams from these faecal pellets were used for each inhalation treatment. Garlic cloves were purchased from the local market in El-Minia Governorate, Egypt. They were then cleaned of any adhered dried material. Each animal was allowed free access to food 50 mg of seeds and cloves daily during the course of the treatment, after fasting for about 12 h.

Experimental animals

Male albino rats, *Rattus norvegicus* (6–8 weeks old, weight 100–120 g) were purchased from the Biological Supply Center, Theodore Bilharz Research Institute, TBRI, Cairo, Egypt and housed under specific pathogen-free conditions and maintained on a 12-h light-dark cycle, with food and water ad libitum. The experiments were conducted according to the ethical norm approved by the Institutional Animal Ethics Committee (IAEC). Animals were classified into four groups (10 animals each). The first group (control) was untreated. The second group was allowed free access to food 50 mg of garlic daily during the course of the treatment, after fasting for about 12 h. The third group was intranasal inhaled with *T. putrescentiae* faeces extract (5 g) daily for 10