

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.

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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 agent¹ 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 bakers⁷ 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 screening²¹ 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 $\times$ and 600 $\times$ 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. $\times$ 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 $25 \pm 1^\circ\text{C}$ 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

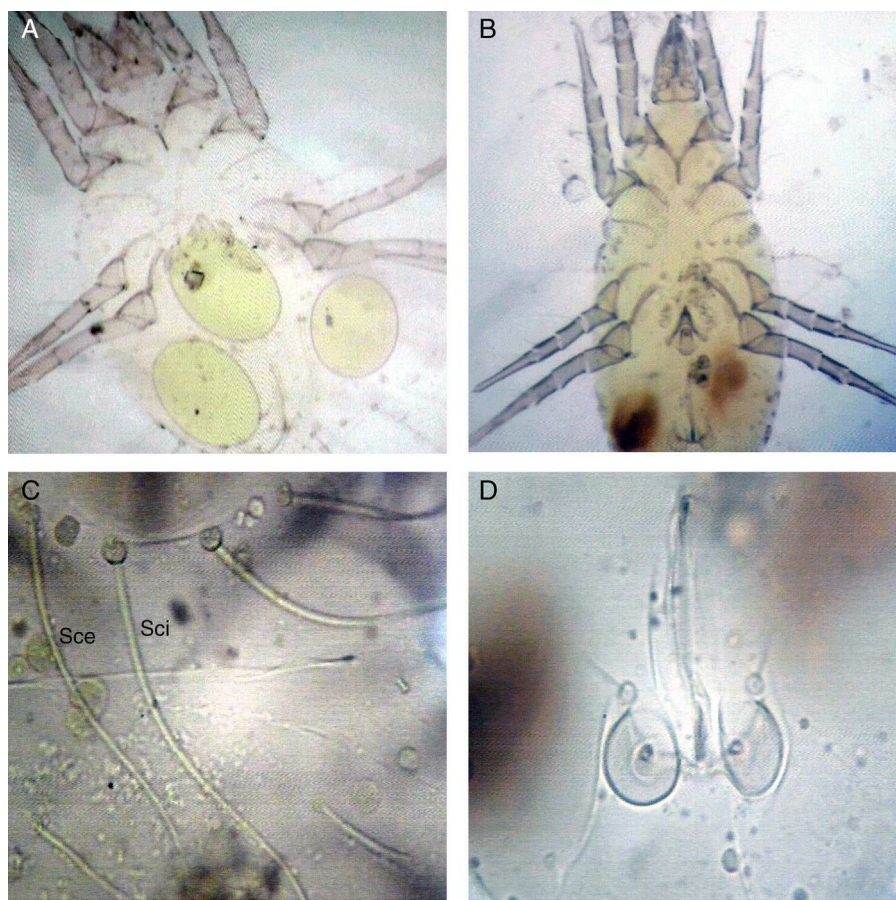


Figure 1 *Tyrophagus putrescentiae*, (A) female whole mount; (B) male whole mount; (C) dorsal view with Sci seta longer than Sce seta; (D) ventral view of male with two anal suckers.

consecutive days. The fourth group inhaled as in the third group and allowed free access to food as in the second group.

Cytokine and immunoglobulin in serum, 72 h after the last treatment, ten animals from each group were sacrificed under chloroform anaesthesia. Blood samples were taken and centrifuged at 3000 rpm for 30 min. Sera were removed and kept at -20°C for the estimation of the cytokines (IFN- γ and IL-4) and IgE antibody levels by ELISA using kits purchased from R&D Systems (Minneapolis, MN, USA).

Statistical analysis

Data were analysed using SPSS program version 13.0. Statistical analysis of the obtained data was performed using one way analysis of variance (ANOVA) test followed by least square differences (LSD) analysis for comparison between means. Results were expressed as mean $\pm$ standard error (SE). Values of $P < 0.05$ were considered statistically significant, while values of $P > 0.05$ were considered statistically non-significant.

Results

Mass rearing of *Tyrophagus putrescentiae*

This species belongs to the family Acaridae. Its dorsal Setae Sci is longer than Sce. In addition, the femur of male

forelegs is not enlarged and without a ventral conical process. Females do not have anal suckers that are present in males (Fig. 1).

Among 13 different media tested for mass rearing of *T. putrescentiae* mites, statistical analysis of the obtained data showed a significant difference ($P < 0.05$) in mite population recorded by wheat germ, roomy cheese, nido, egg yolk and potatoes media compared to those recorded by fish meal, copra, egg albumin, chicken stocks, pollens, bean, human hair and scales and yeast extract (Fig. 2).

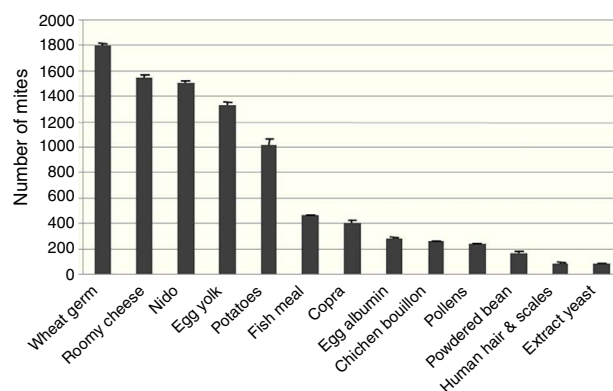


Figure 2 Comparative mass rearing of *T. putrescentiae* under different culture media for two months.

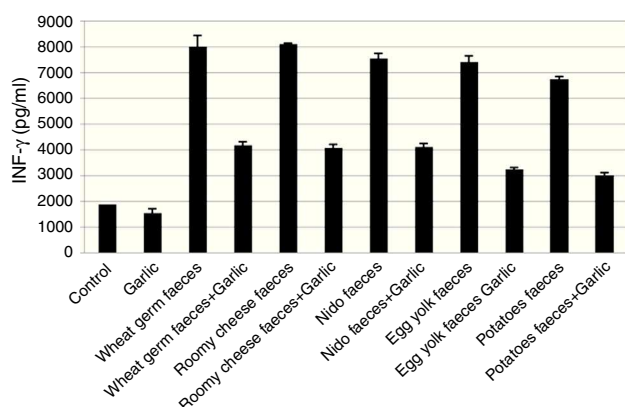


Figure 3 Changes in INF- γ levels in male albino rats inhaled with *T. putrescentiae* mite faeces and treated with garlic.

Quantification of INF- γ

The INF- γ ELISA value demonstrated that the garlic-treated group (1556.47 ± 179.452 pg/ml) was lower than the control group (1885.88 ± 003.867 pg/ml). The groups treated with *T. putrescentiae* faeces produced by wheat germ (8030.29 ± 422.930 pg/ml), roomy cheese (8113.06 ± 036.869 pg/ml), nido (7554.91 ± 186.849 pg/ml), egg yolk (7430.97 ± 214.261 pg/ml) and potatoes (6748.63 ± 102.913 pg/ml) were higher than the groups treated with garlic in addition to the above five diets (4201.97 ± 141.100 pg/ml, 4101.43 ± 110.254 pg/ml, 4129.77 ± 147.799 pg/ml, 3275.37 ± 38.531 pg/ml, 3022.40 ± 105.782 pg/ml, respectively) (Fig. 3). This study also showed that there is a significant difference in INF- γ level between garlic-treated group and either the native or faeces-treated group ($P < 0.05$).

Quantification of IL-4

Results of ELISA in Fig. 4 showed that IL-4 recorded 567.07 ± 07.929 pg/ml in control group. The inhaled group with *T. putrescentiae* faeces counted high level of IL-4 (812.51 ± 22.897 pg/ml). In contrast, the groups treated with *T. putrescentiae* faeces produced by wheat germ (231.12 ± 03.448 pg/ml), roomy cheese (196.90 ± 05.448 pg/ml),

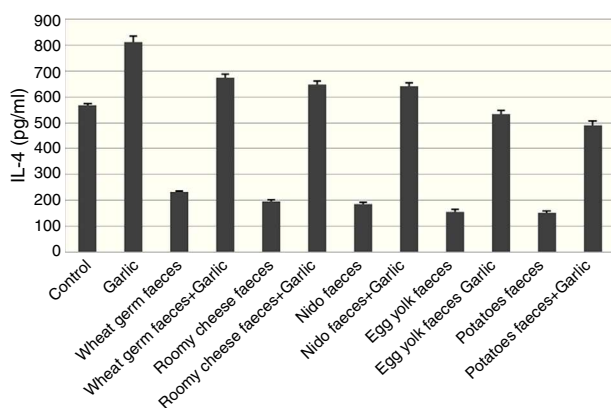


Figure 4 Changes in IL-4 levels in male albino rats inhaled with *T. putrescentiae* mite faeces and treated with garlic.

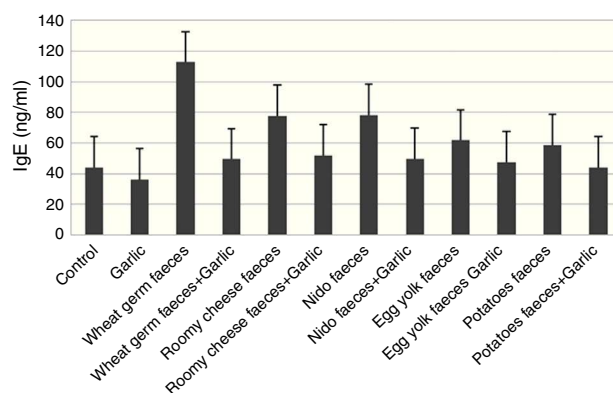


Figure 5 Changes in IgE levels in male albino rats inhaled with *T. putrescentiae* mite faeces and treated with garlic.

nido (187.43 ± 05.339 pg/ml), egg yolk (154.51 ± 10.097 pg/ml) and potatoes (150.80 ± 08.091 pg/ml) are lower than the groups treated with garlic in addition to the above five diets (674.22 ± 13.834 pg/ml, 645.90 ± 15.277 pg/ml, 640.54 ± 13.643 pg/ml, 532.48 ± 15.371 pg/ml, 489.12 ± 17.354 pg/ml, respectively) (Fig. 4). Statistical analysis showed a significant difference in IL-4 between garlic-treated group and either control or faeces-treated group, $P < 0.05$.

Quantification of IgE

In contrast to IL-4, ELISA tests of IgE showed that the garlic-treated group (36.30 ± 0.427 ng/ml) is lower than the control group (44.28 ± 0.400) and the groups treated with *T. putrescentiae* faeces produced by wheat germ (112.87 ± 2.798 pg/ml), roomy cheese (77.71 ± 7.646 pg/ml), nido (78.46 ± 5.449 pg/ml), egg yolk (61.90 ± 4.374 pg/ml) and potatoes (58.84 ± 3.213 pg/ml) are higher than the groups treated with garlic in addition to the above five diets (49.51 ± 3.642 pg/ml, 51.91 ± 1.954 pg/ml, 49.87 ± 1.691 pg/ml, 47.65 ± 1.241 pg/ml, 44.19 ± 1.525 pg/ml, respectively) (Fig. 5). Statistical analysis showed that there is a significant difference between the garlic-treated group and either the control or faeces-treated group ($P < 0.05$).

Discussion

T. putrescentiae mites are known to cause hypersensitivity reactions and asthma.¹⁰ Selection of the culture media is one of the very important factors for successful mass rearing of house dust/storage mites.²⁶ The mite problems described above can have severe effects on the health.¹⁵ The following points must be taken carefully to prepare a specific vaccine against house dust mite bronchial asthma, perennial rhinitis or allergic dermatitis. These are: (i) identification of the local house dust mites, (ii) determination of the sensitising mite allergen and this necessitates isolation of the identified house dust mites extracts, and (iii) identification of the specific house dust mite extract to which patients give positive reaction.

Mass rearing of *T. putrescentiae* was carried out, since this species has proven to be the second most important

allergic mite in Egypt²² as well as in other places such as Sydney and New Guinea.²⁷ It was also recorded in the three governorates with high numbers. In this study the best diet was the wheat germ by which 1881 mites were gained. The second effective diet (cake) gave 1575.9 mites. Roomy cheese gave 1539.7 mites. Also nido, egg yolk and egg albumin gave a number of mites greater than one thousand. On the other hand, potatoes, fish meal, copra, chicken bouillon, pollens, powdered bean, human hair and scales and yeast extract gave a number of mites lower than 500 mites.

Similar and other diets such as powdered animal food;²⁸ (yeast, clover seed, ground nuts, linseed, maize germ, peas and rice);²⁹ (mixture of ground and dried oil liver);³⁰ a mixture of human skin scales and yeast, monosaccharides, disaccharides and polysaccharides;³¹ wheat germ;²⁷ and a mixture of roomy cheese, dried egg yolk and yeast.²² Green and Woodcock mentioned that the type of food ingested can markedly affect the fecundity, duration of development, maturity, longevity and age structure of a *T. putrescentiae* population.²⁷

In addition to the mite mass rearing, we have investigated whether the faecal antigen is important in the development of asthma or not, where mite faecal pellets break up into many small pieces and combine with dust particles to form an allergic dust.³²

The potential of certain pollutants to elicit allergic airway disease has been established in both experimental models and in humans.³³ Short exposure (10 days) to HDM alone or to HDM and OVA resulted in equivalent levels of airway inflammation due to a robust inflammatory response in the lung. Moreover, it has been recently shown that intranasal administration of HDM daily for 10 days induces allergic sensitisation and Th2 airway inflammation, both of which are substantially reduced by concurrent treatment with anti-M-CSF antibodies.³⁴ This prompted us to expose the tested animals for about 10 days.

Allergic disorders have been rising in recent years. It has been demonstrated that IgE antibodies play an important role in mediating type I hypersensitivity in humans.³⁵ In both food and inhalant allergy it is accepted that food- or HDM-specific IgE binds to high-affinity FcεRI on mast cells, basophiles, macrophages, and dendritic cells, as well as to low-affinity FcεRII on macrophages, monocytes, lymphocytes, eosinophils, and platelets.³⁶ When mite allergens penetrate mucosal barriers of the gastrointestinal or respiratory tract and contact IgE antibodies bound to mast cells or basophiles, histamine and other mediators that induce symptoms of immediate hypersensitivity are released.³⁷ Other studies have also suggested that cleavage of CD23 (low-affinity IgE receptor) may promote and enhance an IgE immune response.³⁸

Another factor to consider is cytokine production. Indeed, it has been suggested that HDM may privilege the generation of a Th2-polarised response by modulating the balance between IL-4 and IFN-γ.³⁹ In addition, various dust mite allergens act on bronchial epithelial cells in vitro to elicit the production of a number of cytokines.⁴⁰

IL-4 is a key anti-inflammatory cytokine and plays an important role in the cytokine network and in the Th1/Th2 balance. It is generated by helper T cells, and possibly in the early adaptive immune response by mast cells and

basophils.³⁵ In this study, the low level of IL-4 in the faeces-treated group may be due to the negative feedback that is exerted by histamine itself to inhibit IL-4.³⁷

Thus, this study tended to measure the levels of INF-γ, L-4 and IgE that are involved in allergic disease. Repeated exposure to *T. putrescentiae* allergic faeces caused an increase in both INF-γ and IgE, and a decrease in IL-4. The high level of both INF-γ and IgE, and the low level of IL-4 may participate in airway disorder. After garlic feeding, the increase of IL-4 and the decrease of both INF-γ and IgE were maintained, and this may play an important role in the airway remodelling processes. The immunomodulatory effects on INF-γ, IL-4 and IgE balance were tested by others.^{14,41}

Garlic in small amounts applied as a food condiment does not usually pose an important risk, except for rare specific allergic reactions in hypersensitive people.⁴² In contrast, this study showed that some allergic disorder could be modulated by garlic.

In conclusion, these data demonstrated that wheat germ, roomy cheese, nido, egg yolk and potatoes media increase the mass rearing of *T. putrescentiae* compared to those recorded by fish meal, copra, egg albumin, chicken stocks, pollens, bean, human hair & scales and yeast extract. Repeated exposure to *T. putrescentiae* allergic faeces caused an increase in both the pro-inflammatory cytokine INF-γ and the main allergic immunoglobulin IgE, and a decrease in the anti-inflammatory cytokine IL-4. The high level of both INF-γ and IgE, and the low level of IL-4 may participate in airway disorder. After garlic treating, the increase of IL-4 and the decrease of both INF-γ and IgE were maintained, and this means that garlic may play an important role in the amelioration of the airway immune response.

Ethical disclosures

Protection of human and animal subjects. The procedures followed were in accordance with the regulations of the responsible Clinical Research Ethics Committee and in accordance with those of the World Medical Association and the Helsinki Declaration.

Confidentiality of data. No patient data appears in this article.

Right to privacy and informed consent. No patient data appears in this article.

Conflict of interest

The author has no conflict of interest to declare.

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