LONG-TERM EFFICACY OF PROTEIN-ENRICHED PEA FLOUR AGAINST TRIBOLIUM CASTANEUM (COLEOPTERA: TENEBRIONIDAE) IN WHEAT FLOUR

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ABSTRACT

Long-term efficacy of the protein-enriched flour of pea (Pisum sativum L. var. Bonneville) in its toxicity, progeny reduction and organoleptic properties was evaluated by combining it with wheat flour and testing the admixture against the red flour beetle, Tribolium castaneum (Herbst). The toxicity and progeny-reducing effects of the wheat flour treated with protein-enriched pea flour were stable for a period of 5 months when stored at 28°C with 75% r.h. Heat treatment destroyed the biological activity of the protein-enriched pea flour containing the active ingredient due to the denaturation of proteins. The organoleptic properties of stored wheat flour were not affected by the treatment with protein-enriched pea flour.

KEYWORDS: Protein-enriched pea flour, Long-term efficacy, Toxicity, Progeny reduction, Organoleptic tests, Tribolium castaneum

INTRODUCTION

Insect pests cause damage to stored grain and processed products by reducing dry weight and nutritional value [18]. Around the world, residual chemical insecticides and fumigation are currently the methods of choice for the control of stored-product insects [20]. Their widespread use has led to some serious problems, including development of insect strains resistant to insecticides [22], toxic residues on stored grain, and health hazards to grain handlers.

Increased public concern over the residual toxicity of insecticides applied to stored products, the occurrence of insecticide-resistant insect strains, and the precautions necessary to work with traditional chemical insecticides stress the usage of botanicals to control stored product insects [19]. Plant natural products with good insecticidal activity are more likely to be used as leads for the synthesis of new insecticides [1]. Yellow split-peas (Pisum sativum L.) mixed with wheat resulted in a marked reduction of survival and reproduction rate of the rice weevil, Sitophilus oryzae (L.) [4] [7]. Bodnaryk et al. found that a protein-rich fraction extracted from peas controlled several stored-product insect pests [2]. Fields et al. observed a significant negative correlation between pea protein concentration and the number of adult S. oryzae in treated wheat kernels [5]. Pea protein treated wheat caused increased movement of S. oryzae out of the grain when exposed for a time period of 24 h [12]. Barley treated with pea flour extract at 0.1% reduced adult numbers of S. oryzae by 93% [8]. Pea protein is repellent to many stored-product insects [10]. The repellency to S. oryzae of milled rice treated with 1% protein-enriched pea flour, and of the untreated control, was 91.2% and 13.8% respectively after 48 h from the introduction of insects [15].

The red flour beetle, Tribolium castaneum (Herbst) is a serious pest in milled products causing heavy loss [13]. So far, no study has been carried out to assess the efficacy of protein-enriched pea flour on the processed food commodity, viz., wheat flour, on the long-term efficacy of its toxicity, progeny reduction and impact on organoleptic properties. Hence, this paper would help to understand the importance of protein-enriched pea flour in offering protection to milled products from insect damage especially in flour mills.

MATERIAL AND METHODS

Test product

Whole pea seeds (Pisum sativum var. Bonneville, a wrinkled seeded pea variety), obtained from Bangalore, Karnataka state, India, were ground to a fine powder. Fractions of 53 μ size were obtained from the whole flour by the air classification process of Wright et al. [21] with the use of an electronic sieve shaker that passed the whole-pea flour through different sized sieves. Protein-rich fractions were purified using ammonium sulphate precipitation [17]. 100 g of the pea flour obtained by the air classification process were dissolved in one liter of 0.2 M phosphate buffer (pH = 7.5), kept overnight and passed through filter paper. 611 g of solid ammonium sulphate was added per liter of solution, stirred well and kept overnight at 4° C. It was then centrifuged at 10,000 rpm for 20 min and the precipitate was air dried and used. The protein-enriched pea flour obtained by the ammonium sulphate precipitation method contained 90% protein content as estimated by the method of Lowry et al. [11].

Test insect

Local strains of Tribolium castaneum (Herbst) that were mass cultured on wheat flour were used. They were maintained at ambient laboratory temperature of 28–32°C and 75-80% r.h.

Long-term efficacy tests

Protein-enriched pea flour was mixed with wheat flour at concentrations of 0%, 0.001%, 0.01%, 0.1% and 1% w/w basis. Part of the treated flour was maintained at 28°C with 75% r.h. for 6 months to evaluate the long-term efficacy of protein-enriched pea flour in its toxic effect. This was designated as aged protein-treated flour. For comparison, another set of treated flour without any time delay was also evaluated for its toxicity. This was designated as new protein-treated flour. The long-term efficacy of the protein-enriched pea flour was tested in terms of its impact on the mortality and progeny reduction of T. castaneum. Wheat flour samples of 100 g quantity were used for all the treatments. Both the aged and new protein-treated wheat flour were placed separately in plastic containers (9 cm high and 7 cm dia). For adult mortality trials, 20 unsexed adults of T. castaneum were introduced into each container and the containers were covered with muslin cloth. Mortality of adults was then determined after 2 and 4 weeks.

For assessing the long-term efficacy of protein-enriched pea flour in reducing the progeny, both the aged and new protein-treated wheat flour were placed separately in the plastic containers. Twenty unsexed adults of T. castaneum were introduced into each container, and the containers were covered with muslin cloth. After 2 weeks, the adult insects were removed from all the containers. The containers were allowed to stand for an additional 5 weeks before counting the offspring adults. Four replications were maintained for each treatment.

The studies were conducted at a room temperature of 28°C with 75% r.h.

Effect of heat

Protein-enriched pea flour which was heated to 100°C for 30 min was used to assess the effect of heat on its toxicity against T. castaneum. The toxic effects of non-heated protein-enriched pea flour was also evaluated for comparison. Both the heated and non-heated protein-enriched pea flour were mixed with wheat flour at concentrations of 0%, 0.001%, 0.01%, 0.1% and 1% w/w basis. A quantity of 100 g of wheat flour was used for all the treatments. Both the non-heated and heated protein-enriched pea flour treated wheat flour were placed separately in the plastic containers (9 cm high and 7 cm dia). Twenty unsexed adults of T. castaneum were introduced into each container and the mortality was determined after 2 and 4 weeks. Four replications were maintained for each treatment. The studies were conducted at a room temperature of 28°C with 75% r.h.

Organoleptic tests

Wheat flour treated with protein-enriched pea flour at 1% concentration was tested for organoleptic qualities such as taste, smell and palatability by following the ISI method [9]. For this purpose, 1 kg of wheat flour treated with 10 g of protein-enriched pea flour, which was stored for 3 months was used. Observations on attributes such as the appearance, taste, texture, flavor and acceptability of the treated wheat flour were recorded and compared with those of untreated control. The scores were given by a panel of 20 persons for each of the different attributes based on the ranking scale.

Statistical analysis

The data pertaining to the observations in the laboratory were analysed in a completely randomized design. The mean values of the experiments were separated using Duncan's Multiple Range Test [6].

RESULTS AND DISCUSSION

Insecticidal activity of protein-enriched pea flour was reduced after treated wheat flour was maintained at 28°C with 75% r.h. for 6 months. New protein-treated wheat flour at 1% concentration caused adult T. castaneum a mortality of 36.2% and 83.5% after 2 and 4 weeks exposure, respectively (Table 1). At the same concentration, aged protein-treated wheat flour resulted in 28.6% and 46.3% mortality after an exposure period of 2 and 4 weeks respectively. Studies conducted with pea extracts suggest that protein-rich fraction acts as a toxicant [2] [15]. Data presented in Table 2 show comparative results of the effect of new and aged protein-treated wheat flour on the progeny of T. castaneum. Progeny reduction effect of treated wheat flour maintained at 28°C with 75% r.h. for 6 months was reduced compared to new protein-treated wheat flour. The average emergence of adult T. castaneum from 0.1% new protein-treated flour was 62.2, while it was 97.4 in the case of aged protein-treated flour at the same concentration. Among the tested concentrations, new protein-treated wheat flour at 1% concentration was more pronounced in suppressing the emergence of offspring adults. Progeny reduction of T. castaneum could be due to ovicidal and larvicidal action or inhibition of oviposition. Exposure of T. castaneum to flour disks prepared with pea protein indicated that consumption of flour disks by T. castaneum decreased with increased concentration of the pea protein-rich fraction [14]. Protein-enriched pea

Table 1 Percentage adult mortality (mean ± S.E.) of *Tribolium castaneum* exposed to wheat flour treated with aged and new protein-enriched flour of Bonneville pea after 2 and 4 weeks of insect introduction

Concentration	2 weeks		4 weeks	
	Aged[1]	New[2]	Aged[1]	New[2]
0	0 Ad	0 Ad	3.7 ± 0.5 Ad	3.8 ± 0.7 Ae
0.001%	0 Ad	0 Ad	5.0 ± 0.4 Bd	12.5 ± 1.6 Ad
0.01%	6.5 ± 0.3 Bc	13.7 ± 2.3 Ac	11.2 ± 1.8 Bc	28.6 ± 2.5 Ac
0.1%	13.8 ± 1.1 Bb	20.0 ± 2.6 Ab	23.7 ± 2.7 Bb	47.9 ± 2.1 Ab
1%	28.6 ± 2.4 Ba	36.2 ± 0.7 Aa	46.3 ± 1.5 Ba	83.5 ± 1.9 Aa

Values are means of four observations.
[1]Protein-enriched pea flour mixed wheat flour was maintained at 28°C with 75% r.h. for 6 months before use.
[2]Protein-enriched pea flour was mixed with wheat flour and immediately used.
In a column, means followed by the same letter (lower case) and in a row, means followed by the same letter (upper case) are not significantly different by Duncan's multiple range test ($P = 0.05$)
(analysed separately for the different time duration).

Table 2 Number of progeny developed as adults (mean ± S.E.) of *Tribolium castaneum* produced in wheat flour treated with aged and new protein-enriched flour of Bonneville pea over a period of 7 weeks

Concentration	Aged[1]	New[2]
0	188.7 ± 2.7 Ad	189.5 ± 2.5 Ae
0.001%	185.6 ± 2.9 Bd	176.3 ± 1.8 Ad
0.01%	141.2 ± 1.6 Bc	119.0 ± 1.5 Ac
0.1%	97.4 ± 1.1 Bb	62.2 ± 2.2 Ab
1%	52.3 ± 1.4 Ba	7.1 ± 1.3 Aa

Values are means of four observations.

[1]Protein-enriched pea flour mixed wheat flour was maintained at 28°C with 75% r.h. for 6 months before use.

[2]Protein-enriched pea flour was mixed with wheat flour and immediately used.

In a column, means followed by the same letter (lower case) and in a row, means followed by the same letter (upper case) are not significantly different by Duncan's multiple range test ($P = 0.05$).

Table 3 Percentage adult mortality (mean ± S.E.) of *Tribolium castaneum* exposed to wheat flour treated with non-heated and heated protein-enriched flour of Bonneville pea after 2 and 4 weeks of insect introduction

Concentration	2 weeks		4 weeks	
	Non-heated	Heated*	Non-heated	Heated*
0	0 Ad	0 Ab	3.1 ± 0.4 Ae	1.2 ± 0.6 Ac
0.001%	0 Ad	0 Ab	11.5 ± 0.7 Ad	1.4 ± 0.3 Bc
0.01%	12.9 ± 0.5 Ac	0 Bb	27.3 ± 2.2 Ac	3.8 ± 1.4 Bbc
0.1%	19.8 ± 1.7 Ab	1.3 ± 1.1 Bb	46.6 ± 2.6 Ab	8.7 ± 1.8 Bb
1%	35.4 ± 1.9 Aa	5.0 ± 1.6 Ba	81.9 ± 2.3 Aa	22.1 ± 2.5 Ba

Values are means of four observations.

* Protein-enriched pea flour which was heated to 100°C for 30 min was used.

In a column, means followed by the same letter (lower case) and in a row, means followed by the same letter (upper case) are not significantly different by Duncan's multiple range test ($P = 0.05$)
(analysed separately for the different time duration).

Table 4 Scores (mean ± S.E.) given by 20 persons for the cooked product (rhoti) prepared by using wheat flour after treatment with 1% protein-enriched flour of Bonneville pea for 3 months

Attribute	Control (untreated)	Treated
Appearance	3.9 ± 0.5	4.0 ± 0.1
Taste	2.7 ± 0.3	3.1 ± 0.8
Texture	2.9 ± 0.7	3.2 ± 0.5
Flavor	2.8 ± 0.4	3.0 ± 0.2
Acceptability	3.7 ± 0.6	4.1 ± 0.9

Raking scale for attributes
0 = very poor, 1 = poor, 2 = fair, 3 = good, 4 = very good, 5 = excellent

flour would have interfered with the process of feeding and thus oviposition by the stored-product insects as eating is an essential precursor for laying eggs.

Toxic and progeny-reducing effects of protein-enriched pea flour declined after treated wheat flour was maintained at 28°C with 75% r.h. for 6 months. For all biological products, there would be an expiry point until which, the activity remains similar. A decline in the toxicity and progeny reduction property of protein-enriched pea flour after a certain time period could be a consequence of enzymatic degradation of the active ingredient present in the protein-enriched pea flour.

Heating the protein-enriched pea flour greatly reduced the insecticidal activity against T. castaneum. For instance, non-heated protein admixed with wheat flour at 1% concentration caused 35.4 and 81.9% mortality of T. castaneum adults after 2 and 4 weeks exposure, respectively. At the same concentration, heated protein-treated wheat flour caused T. castaneum, a mortality of 5.0% and 22.1% after an exposure period of 2 and 4 weeks, respectively (Table 3).

Heating proteins can result in drastic effects on their biological activity due to denaturation. As they are heated, the atoms vibrate more rapidly and the delicately arranged secondary, tertiary and quaternary structures are lost [3]. The chains usually assume more randomly arranged structures with fewer charged groups to the outside of the molecules, which greatly reduces their solubility resulting in the loss of biological activity. Heat treatment destroyed the biological activity of the protein-enriched pea flour containing the effective ingredient. Thus, the active ingredient could be defined as being heat-sensitive.

Any treatment of stored products in addition to the protection offered to the commodity, should not affect the organoleptic characteristics and acceptability of the treated material [16]. Results of such a study involving a group of 20 persons are presented in Table 4. Wheat flour treated with protein-enriched Bonneville pea flour at 1% concentration, which is the effective concentration among the various treatments, did not affect the appearance, taste, flavour, texture and overall acceptability. Since the treatment did not affect the organoleptic properties of the commodity and in addition, offered protection, it could be inferred that the protein-enriched pea flour be conveniently used for controlling insect infestation in stored food commodities, thereby protecting the quality and quantity.

Natural insecticides are highly useful for the safe storage of food commodities. Protein-enriched pea flour seems to act as a good protectant for processed stored products from insect damage as it possess excellent toxic and progeny reduction property, hence, it could effectively fit well in the integrated pest management of stored-product insects.

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