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Protein-enriched pea flour extract protects stored milled rice against the rice weevil, *Sitophilus oryzae*

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Abstract

Studies were conducted to evaluate the effect of a protein-enriched pea (*Pisum sativum* var. Bonneville) flour extract against the rice weevil, *Sitophilus oryzae* in its repellency, toxicity, effect on fecundity, stability and sensory properties. Milled rice admixed with pea flour extract at 1% concentration significantly repelled *S. oryzae*. Mortality of *S. oryzae* was found to increase and fecundity was markedly suppressed, in rice treated with 1% pea flour extract. The toxicity and reproductive effects of the pea protein-enriched rice were found to be stable for a period of 5 months. The sensory characteristics of stored rice when eaten were not affected by the treatment with pea flour extract. This study indicates that the protein-enriched flour extract obtained from the Bonneville pea may be feasible to protect stored milled rice from insect attack.

Keywords: repellency, toxicity, fecundity, stability, sensory properties

Introduction

Rice is the most important food crop for more than half of the world's population. Losses in rice storage due to insect pests affect food availability for a large number of people. Milled rice is attacked by various insect pests during storage (Cogburn 1980). The rice weevil, *Sitophilus oryzae* L. is a major pest of stored rice in India. Use of chemical insecticides can result in harmful residues in foodstuff and development of resistance in the target insect populations (Zettler and Cuperus 1990). As the mixing of chemicals with milled rice is not recommended, the use of safer materials of botanic origin offers a promising alternative for the protection of milled rice against insect pests.

The insecticidal activity of many plant derivatives against several stored-product pests has been demonstrated (Weaver and Subramanyam 2000). Legume seeds contain a wide range of allelochemicals with toxic and deterrent effects against insect pests (Bell 1978). Pea protein is repellent to several stored-product insects (Fields *et al*. 2001; Kumar *et al*. 2004) and toxic (Bodnaryk *et al*. 1999). The challenge is to develop new or enhanced technologies to protect stored food commodities. The present studies were therefore undertaken to evaluate the effectiveness of protein-enriched pea flour extract to protect milled rice against *S. oryzae*.

Materials and Methods

Test materials

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*. (1984) 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 sulfate precipitation (Sadasivam and Manickam 1996). 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 sulfate 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 sulfate precipitation method contained 90% protein content as estimated by the method of Lowry *et al*. (1951).

Local strains of *Sitophilus oryzae* (L.) that were mass cultured on whole sorghum grain were used. They were maintained at ambient laboratory temperature of 28 –32° C and 75-80% RH.

Repellency tests

The cup bioassay technique (Kumar *et al*. 2004) was used to evaluate the repellent effect of the pea flour extract when added to milled rice against *S. oryzae*. A quantity of 200 g of milled rice was treated with the pea flour protein extract at concentrations of 0%, 0.001%, 0.01%, 0.1% and 1% on a w/w basis. The treated rice was placed in a covered container perforated with holes large enough to allow weevils to pass through. 20 unsexed adults of *S. oryzae* were placed on the rice in the center of the perforated container. The repellency of the protein-enriched pea flour was measured in terms of the percentage of insects moving out of the container,

away from the treated rice. They were trapped in a tray below the container. Comparisons were made between weevils exposed to treated and untreated rice. The number of trapped insects was determined at 1, 12, 24, 36 and 48 h after the introduction of the insects. The studies were conducted at a room temperature of 28° C and 75% RH. Morgan et al. (1998) showed that if a test material is repellent, the insects move away and are trapped.

Toxicity and fecundity-reduction

Pea flour protein extract was mixed with milled rice at concentrations of 0%, 0.001%, 0.01%, 0.1% and 1% w/w basis. A quantity of 100 g of milled rice was used in all treatments. The treated rice was placed in plastic containers (9 cm high and 7 cm diameter) and 20 unsexed adults of *S. oryzae* were introduced into each container, and the containers were covered with muslin cloth. Observations on the mortality of adults were carried out at 1, 2, 3 and 4 weeks after the introduction of the insects.

For evaluating the effect of pea flour protein extract on fecundity of *S. oryzae*, the rice treated with pea extract was placed in the plastic containers. Ten unsexed adults of *S. oryzae* were introduced into each container, and the containers were covered with muslin cloth. After 2 weeks, the introduced adult insects were removed from the containers. Five to seven weeks later adult offspring were counted. The studies were conducted at a room temperature of 28° C and 75% RH.

Stability

Pea flour protein extract was mixed with milled rice at concentrations of 0%, 0.001%, 0.01%, 0.1% and 1% w/w basis. The treated rice was maintained at 28° C and 75% RH for 6 months before use to evaluate the stability of the toxicity of the pea extract. These treatments are designated here as aged protein-treated rice. As a control, rice was treated with freshly prepared pea flour extract. These treatments are designated as new protein-treated rice. 100 g of milled rice was used for all the treatments. Both the aged and new protein-treated rice were placed separately in plastic containers (9 cm high and 7 cm diameter). 20 unsexed adults of *S. oryzae* were then introduced into each container and the containers were covered with muslin cloth. Mortality of adults were determined after 2 and 4 weeks.

To assess the stability of aged pea flour protein extract in reducing fecundity, both the aged and new protein-treated rice were prepared as mentioned above and were placed in plastic containers. Ten unsexed adults of *S. oryzae* were introduced into each container, and the containers were covered with muslin cloth. After 2 weeks, the introduced adult insects were removed from all the containers. Five to seven weeks later adult offspring were counted. The studies were conducted at a room temperature of 28° C and 75% RH.

Sensory tests

Milled rice treated with pea flour protein extract at 1% concentration was tested for sensory qualities such as taste, smell and palatability by following the ISI method (1983). For this purpose, 1 kg of rice treated with 10 g of pea flour extract, which was stored for 3 months was used. Observations on attributes such as the appearance, taste, texture, flavor and acceptability of the treated rice 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 analyzed in a completely randomized design. The mean values of the experiments were separated using Duncan's Multiple Range Test (Gomez and Gomez 1984).

Results and Discussion

Bonneville pea flour protein extract at 1% concentration caused significantly increased emigration of *S. oryzae* from the milled rice (Table 1). The repellent effect of pea flour extract was reflected by the fact that the number of insects in treated rice decreased as the concentration increased. Among the tested concentrations, protein-enriched pea flour treated milled rice at 1% w/w basis exposed for a period of 48 h significantly repelled *S. oryzae* which indicates that 1% concentration would be highly effective in preventing the infestation by *S. oryzae*.

Bodnaryk *et al*. (1999) reported in their studies that pea starch is not repellent but that pea protein possessed repellent activity against *S. oryzae*. Pea protein treated wheat resulted in the increased movement of *S. oryzae* out of the grain when exposed for a period of 24 h (Mohan and Fields 2002). The repellency to *S. oryzae* of

Table 1. Repellency of *Sitophilus oryzae* by rice treated with Bonneville pea flour extract.

Concentration	% repellency at different time intervals (mean ± S.E.)				
	1 h	12 h	24 h	36 h	48 h
0	0 Db	3.9 ± 0.0 Cd	6.2 ± 2.5 Cc	10.0 ± 0.0 Bd	13.8 ± 2.5 Ae
0.001%	1.2 ± 0.0 Cb	8.8 ± 2.5 Bc	11.2 ± 2.5 Bc	11.7 ± 2.5 Bd	21.3 ± 2.5 Ad
0.01%	5.0 ± 0.0 Ea	15.0 ± 0.0 Dbc	25.0 ± 0.0 Cb	37.5 ± 2.8 Bc	45.0 ± 0.0 Ac
0.10%	7.5 ± 2.8 Ea	23.7 ± 2.5 Db	48.8 ± 2.5 Ca	63.7 ± 2.5 Bb	76.3 ± 2.5 Ab
1%	10.0 ± 0.0 Ea	36.3 ± 2.5 Da	53.7 ± 2.5 Ca	75.0 ± 0.0 Ba	91.2 ± 2.5 Aa

Values are the means of 4 observations with 20 weevils in each.

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 2. Mortality of *Sitophilus oryzae* exposed to rice treated with Bonneville pea flour extract at different time period after insect introduction.

Concentration	% mortality of insects (mean ± S.E.)			
	1 week	2 weeks	3 weeks	4 weeks
0	0 Ad	0 Ad	0 Ae	0 Ae
0.001%	0 Cd	1.3 ± 0.0 Cd	13.8 ± 2.5 Bd	22.5 ± 2.8 Ad
0.01%	5.0 ± 0.0 Dc	15.0 ± 0.0 Cc	28.7 ± 2.5 Bc	41.2 ± 2.5 Ac
0.10%	13.7 ± 2.5 Db	33.8 ± 2.5 Cb	52.5 ± 2.8 Bb	78.7 ± 2.5 Ab
1%	31.2 ± 2.5 Ca	94.2 ± 2.5 Ba	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa

Values are means of four observations.
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. Progeny of *Sitophilus oryzae* developed from rice treated with aged and new pea flour extract.

Concentration	Number of offspring after 7 weeks (mean ± S.E.)	
	Aged[1]	New[2]
0	210.2 ± 2.0 Ae	209.7 ± 1.7 Ae
0.001%	190.3 ± 2.9 Bd	168.8 ± 2.5 Ad
0.01%	148.8 ± 2.8 Bc	112.0 ± 1.5 Ac
0.10%	96.5 ± 1.9 Bb	41.7 ± 0.5 Ab
1%	61.8 ± 1.8 Ba	2.3 ± 0.9 Aa

Values are means of four observations.
[1]Protein-enriched pea flour mixed rice was maintained at 28°C with 75% r.h. for 6 months before use.
[2]Protein-enriched pea flour was mixed with rice 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 4. Mortality of *Sitophilus oryzae* exposed to rice treated with aged and new pea flour extract 2 and 4 weeks after insect introduction.

Concentration	% mortality of insects (mean ± S.E.)			
	2 weeks		4 weeks	
	Aged[1]	New[2]	Aged[1]	New[2]
0	0 Ad	0 Ad	0 Ae	0 Ae
0.001%	0 Ad	1.3 ± 0.0 Ad	8.7 ± 2.5 Bd	22.5 ± 2.8 Ad
0.01%	7.5 ± 2.8 Bc	15.0 ± 0.0 Ac	21.3 ± 2.5 Bc	41.2 ± 2.5 Ac
0.10%	21.2 ± 2.5 Bb	33.8 ± 2.5 Ab	46.2 ± 2.5 Bb	78.7 ± 2.5 Ab
1%	48.8 ± 2.5 Ba	94.2 ± 2.5 Aa	56.2 ± 2.5 Ba	100.0 ± 0.0 Aa

Values are means of four observations.
[1]Protein-enriched pea flour mixed rice was maintained at 28°C with 75% r.h. for 6 months before use.
[2]Protein-enriched pea flour was mixed with rice 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).

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. A significant negative correlation was observed between pea flour extract concentration and the number of adult *S. oryzae* found in treated milled rice, which is in accordance with the results reported by Fields *et al*. (2001). Barley treated with pea flour extract at 0.1% reduced adult numbers of *S. oryzae* by 93% (Hou and Fields 2003).

Rice treated with the pea flour extract was found to be very effective in causing mortality and reducing fecundity of *S. oryzae*. Milled rice treated with pea flour extract at 1% concentration resulted in 100% mortality of *S. oryzae* in 3 weeks (Table 2). The mortality observed could be a consequence of both toxic and antifeedant effects. Significant reductions were found in fecundity

Table 5. Scores (mean ± S.E.) given by a group of 20 persons for the cooked rice samples after treatment with 1% Bonneville pea flour extract for 3 months.

Attribute	Control (Untreated)	Treated
Appearance	4.0 ± 0.6	3.9 ± 0.3
Taste	2.9 ± 0.7	3.0 ± 0.6
Texture	2.8 ± 0.4	3.1 ± 0.3
Flavour	2.7 ± 0.5	3.2 ± 0.4
Acceptability	3.8 ± 0.6	4.1 ± 0.5

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

during a period of 7 weeks in milled rice treated with all concentrations of pea flour extract compared to the control (Table 3). This obviously would greatly suppress the population of *S. oryzae*. The effect of the aged pea flour extract on fecundity was less than that seen on rice treated with freshly prepared pea extract. The effect of the pea flour extract on fecundity is most likely due to reduced feeding (Pretheep-Kumar and Mohan, 2004) that results in decreased egg production.

The toxic and fecundity-reducing effects of pea flour extract declined after treated milled rice was maintained at 28° C and 75% RH for 6 months. Rice treated with freshly prepared protein at 1% concentration caused a mortality of 94.2% and 100.0% after 2 and 4 weeks exposure, respectively (Table 4). At the same concentration, aged protein-treated rice resulted in 48.8% and 56.2% mortality after the same period. Reduction in the biological activity of pea flour extract after a certain time period could be due to enzymatic degradation of the active factors in the extract.

Treatment of food commodities in addition to the protection offered against insects, should not affect the sensory characteristics and acceptability of the treated material (Reddy and Reddy 1987). Protein-enriched flour of Bonneville pea treated rice at 1% concentration did not affect the appearance, flavor, taste, texture and overall acceptability (Table 5).

Treating rice with protein-enriched pea flour offered protection against *S. oryzae* for a longer period compared to other conventional control measures. The protein-enriched pea flour extract treated rice was found to act as a repellent, insecticide, antifeedant and reduced fecundity in *S. oryzae* without making it less acceptable as a food. Protein-enriched pea flour seems to be well suited to protect stored milled rice against insect pests.

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