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Full Length Research Article

Use of Pea Protein Fractions in the Management of the Red Flour Beetle, *Tribolium castaneum* Herbst

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Abstract

Revolutionary advancement in technology has resulted in an increase in food production and also there is a tremendous loss of stored food products annually due to the destruction of them by various storage pests. Thousands of plant products with insecticidal properties have been identified so far. The present study was aimed to test the efficacy of protein fractions of *Pisum sativum* as a potential insecticidal agent in the management and regulation of storage pest, *Tribolium castaneum* as well as its use as an anti-microbial agent. Purified protein fractions of three different concentrations, 0.25g, 0.5g and 1.0g repeated five times, were mixed with the 10g feed, into which five pairs of *Tribolium castaneum* adults from a stock culture were released. The trends in their mortality were observed over a period of 7 days. The results of the study revealed a positive relation between the concentration of protein fractions and the average mortality rate of adult pests. Biochemical analysis of the treated insects also revealed a significant reduction in their metabolic rates. Besides, promising results were also obtained on the assessment of anti-microbial properties exhibited by the same against pathogenic inoculums of *Streptococcus pneumonia, Salmonella typhii* and *E. coli.*

***Key words*:** *Pisum sativum, Tribolium castaneum Streptococcus pneumonia, Salmonella typhii, E. coli,* Anti-microbial agent

Storage pest infestation adversely affect the availability, edibility and wholesomeness of the stored products [1]. Various insect pests cause extensive post-harvest spoilage to the products that accounting for about 10-20% of storage losses. Their presence in the stored grains can go undetected for a long period of time, which will result in greater damage. One of the major feeders commonly found in the storage facilities is the red flour beetle - *Tribolium castaneum* of the order Coleoptera and family Tenebrionoidea. The pest is known to cause substantial losses – quantitatively as well as qualitatively in products like cereals, brans, oil seeds, beans, peas, flour etc. It is considered a cosmopolitan insect, but are more widespread in the tropical regions. Its management and regulation can be done through both chemical and non- chemical methods [2]. The non-selective use of broad spectrum of chemicals has created more problems, than resolving them. Their extensive usage has resulted in the development of increasing pesticide resistance, increased toxicity in food products and the destruction of beneficial organisms [3]. These undesirable consequences have diverted humans towards their replacement with natural pesticides of plant origin. In the present study, the insecticidal and anti-microbial property of the of garden pea - *Pisum sativum* is investigated which is an herbaceous annual of the Fabaceae family. It is an important constituent of human diet for hundreds of years, because of its high nutritional contents. They are fairly low in calories but are good sources of proteins, amino acids and carbohydrate.

MATERIALS AND METHODS

For the evaluation of effectiveness of pea protein fractions against *T. castaneum*, a sufficient number of adult beetles were collected and cultured under optimal living conditions. Whole pea seeds were dried and ground to a fine powder. For the preparation of pea protein fractions, finely powdered pea powder obtained after sieving was purified using Ammonium sulphate precipitation method. The purified pellets were then collected and air dried.

Assessing the insecticidal activity of pea protein

10g each of un-infested broken wheat grains were taken in 16 bottles with one maintained as control. Three concentrations of pea protein fractions - 0.25g, 0.5g, 1.0g were added with each repeating five times. 10 adult beetles were then released into each bottle and their mortality were recorded after every 24 hours and the trends in mortality was analyzed using

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Abbott's formula. The biochemical analysis of treated insects was done after 7 days. The estimation of total DNA content was done by Diphenylamine method, estimation of total proteins was done using Lowry's protein assay and estimation of carbohydrates by Anthrone method.

Antimicrobial property of pea protein

The pathogenic inoculums of *E. coli, S. typhii* and *S. pneumonia* were taken from the stock culture available in the laboratory and were inoculated in a nutrient broth medium for overnight growth. These were swabbed onto the surface of MHA plates labelled appropriately. 50µl, 100µl agar wells were then boarded into labelled petri dishes. Using a sterile micro pipette, the samples were then poured onto each of the wells and incubated for 24 hrs. After the incubation period, the petri plates were taken out and the diameters of zones of inhibition were measure.

RESULTS AND DISCUSSION

Assessing the insecticidal activity of pea protein

Data in (Table 1) shows the average mortality of *T. castaneum* exposed to the three different concentrations of pea protein fractions over a period of 7 days i.e., day 1, day 3, day 5 and day 7. The isolated pea protein when added to the insect diet, had shown significant effects on the mortality rate of insects compared to the untreated control. The insects exposed to 1% concentration of pea protein presented the highest mortality rate with about 72% of average mortality shown at the end of day 7. It is clear from the data that a higher concentration of pea protein fractions up to 1% will effectively reduce the growth and reproduction and eventually kill adult beetles.

Biochemical analysis of treated insects

Estimation of DNA by Diphenylamine method

The total amount of DNA present in the homogenized insects, taken from the protein concentration with highest mortality (1%) were analyzed. (Table 2) shows the absorbance of the samples, both control and test sample at 600nm in a UV/VIS spectrometer. The absorbance of the sample was lower than that of the untreated control, indicating a reduced metabolism in the former.

Table 1 Average insect mortality of different sample concentrations

Pea protein concentration	Average mortality in day 1 (%)	Average mortality in day 3 (%)	Average mortality in day 5 (%)	Average mortality in day 7 (%)
Pea protein 0.25%	4	16	12	14
Pea protein 0.5%	6	26	30	42
Pea protein 1%	10	28	46	72

Table 2 Estimation of DNA, protein, total carbohydrate content in control and treated sample

Sample	DNA		Protein		Total carbohydrate	
	Concentration (µg/mL)	Absorbance at 600 nm (OD)	Concentration (mg/mL)	Absorbance at 660nm (OD)	Concentration (mg/mL)	Absorbance at 630nm
Control	500.022	0.114	2606.108	0.080	1820.521	0.44
Test sample	403.090	0.101	2040.03	0.064	1527.598	0.37

Estimation of protein by Lowry's method

The total protein content of treated insects from the concentration sowing highest mortality was calculated using a UV/VIS spectrometer at 660 nm (Table 2). The absorbance of untreated control was greater than that of the treated insects, which clearly shows a reduction in the metabolism of the latter.

Estimation of carbohydrates by anthrone method

The total carbohydrates of treated insects from concentration with highest mortality was analyzed at 630nm. (Table 2) shows the lower absorbance of the insects from the treated sample than that those from the control. This indicates a lower metabolic rate in the treated insects.

Table 3 Antimicrobial effect of the pea protein fraction

Microorganism	Diameter of inhibitory zone in 50 µl (mm)	Diameter of inhibitory zone in 100 µl (mm)
E. coli	7	0
Streptococcus pneumoniae	18	26
Salmonella typhii	14	14

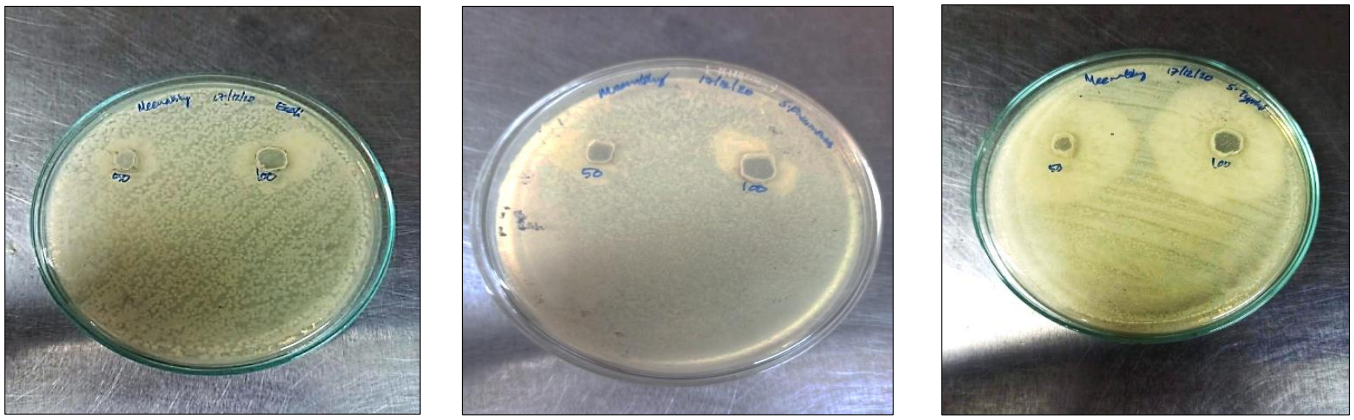

Fig 1 Antimicrobial assay of *Pisum sativum* fractions on (A) *Escherichia coli* (B) *Streptococcus pneumoniae* and (C) *Salmonella typhi*

Antimicrobial property of pea protein

The antibacterial properties exhibited by *Pisum sativum* against pathogenic inoculums of *E. coli, Salmonella typhii* and *Streptococcus pneumoniae* were analyzed. Pea protein solution exhibited promising results and the growth of microbes was considerably affected with the formation of inhibition zones ranging from 7mm to 26mm. (Table 3) shows the different diameters of various zones of inhibition observed. *Pisum sativum* has proved to the highest potency as an inhibitory agent against *Streptococcus pneumoniae*, as a growth inhibitory zone of 26mm was formed.

Control of stored product-pests was one of the major tasks for conservators as the damage inflicted by them is irreversible. A number of insect species pose a threat to various stored produce [4]. The present study was conducted for assessment of the insecticidal as well as the anti-microbial property of the seed extract of *Pisum sativum. Tribolium castanuem* is one of the most common and widespread secondary storage pests around the globe. Various chemical as well as non-chemical methods have been used extensively for their management over the years. Non chemical management of *T. castaneum* and other insect pests was through the spraying of plant-based deterrents and irradiation of the pest [5]. As the use of chemicals have raised the issue of pesticide resistance besides adversely affecting the environment, their replacement with pesticides of a more natural origin is gaining much attention throughout the world.

Protein extracts from legume seeds have previously been proven to possess insecticidal effects. They contain a wide range of allelochemicals with toxic and deterrent effects against insect pests [6]. An admixture of yellow split peas *Pisum sativum* with wheat resulted in a marked reduction in the survival and reproduction rate of *Sitophilus oryzae* [7]. Recently, concentrations as low as 0.01% pea protein were shown to cause adult mortality and reduced reproduction for several stored-product insect pests [8]. The diverse behavioural and physiological effects of the pea fractions, such as repellency shown in the results of the study and reproduction inhibition and oviposition deterrence suggest the presence of a complex of chemicals detrimental to pests.

CONCLUSION

In this present study, 3 different concentrations (0.25%, 0.5% and 1%) of pea protein fractions were mixed with the regular feed of the storage pest *Tribolium castaneum* for 7 days, with each concentration repeating five times. The average insect mortality in day 1, day 3, day 5 and day 7 were analyzed. The pests exposed to 1% concentration of pea protein showed the highest mortality, indicating higher efficacy. Biochemical analysis of the treated insects was analyzed separately. The absorbance of the control sample of all the three tests were greater than that of the test samples indicating a reduction in the metabolism of latter. The antibiotic property of the protein extract against pathogenic inoculums of *E. coli, S. typhii* and *S. pneumoniae* were also analyzed. The results indicated a promising antimicrobial property as the growth of microbes were affected by the extract. This was evident from the formation of inhibition zones with size ranging from 7mm to 26 mm and may be due to the activity of saponins present in the fractions. Thus, the results of the present study have further ascertained the wide potentiality of *Pisum sativum* and validates the use of its protein extracts as effective insecticidal as well as anti-microbial agent.

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