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Effect of Plant oils on biochemical parameters of Potato Tuber Moth *Phthorimaea operculella*

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Abstract
Phthorimaea operculella Zeller (Lepidoptera: Gelechiidae), is one of the most important pest of potato. It is known to cause heavy loss to the potato tubers and the plant as well. Repeated and undue use of conventional chemical insecticides have developed resistance in these insects. Moreover, the chemicals are known to cause ecological imbalance and leave back the residue on the food commodity. This has led to development of alternative strategies to control the pest. Research in this area is progressively appealing consideration as the kingdom of plants is a vast source of active ingredients with insecticidal properties. Many plant products have been explored for their pest control potential. In the present study the essential oils of *Cinnamomum verum*, *Cupressus sempervirens*, *Cymbopogon nardus*, *Emblica Officinalis*, *Rosmarinus officinalis* and *Simmondsia chinensis* were assessed to find their potential as insecticide specially with reference to their effect on the biochemical parameters. The results revealed that the plant oils have significant effect on the biochemical parameters of the pest and can find a place in the control strategy of this major pest of potato.

Keywords: plant oils, *Phthorimaea operculella*, enzymes, protein, carbohydrate

Introduction

Potato, Solanum *tuberosum* L. is a major food crop in many countries of the world. It is one of the most common food constituent in many parts of the world. It occupies the forth place among the most important food crops all over the world, following wheat, rice, and maize (Shady *et al*., 2007) [21]. Potatoes are predisposed to infestation by many insect pests, which reduce yield quantity and quality. Potato tuber moth (PTM), *Phthorimaea operculella* is considered to be one of the most serious insect pests infesting family Solanaceae (Sarhan, 2004 and Mandour *et al*., 2012) [10]. This pest by making irregular tunnels leaves excreta behind and led to a considerable yield loss (Herman *et al*, 2005). It causes serious damage to potato crops in fields and storage (Arnone *et al*, 1998) [1]. Use of conventional chemicals to control this pest has been widely practiced but positively not recommended specially when the application has to be done on the tubers. Resistance to chemical insecticide in PTM has been reported all over the world. Excessive and permanent use of chemical insecticides resulted in the appearance of resistant insects, contamination of ecosystems, and food and feed (Kljajić and Perić, 2004) [6].

The plant products can be used as one alternative methods in this crop protection with one of the main role in the pest management (Pandey *et al*., 1979). It has been proved that secondary metabolites, extracts and essential oils from a large number of plants have insecticidal properties (Shayaa *et al*., 1997; Rice *et al*., 1998) [17]. Keeping in mind the ecofriendly action, the plant origin materials may serve as proper alternative for controlling the potato tuber pest.

The control potential of several plant oils have been studied in order to develop new strategy for the control of *P. operculella* (Kroschel and Koch 1999) [8], still there is enormous scope to explore various plant products as selective control agents for *P. operculella.*

Present study deals the study of effects of the test plant oils viz *Cinnamomum verum*, *Cupressus sempervirens*, *Cymbopogon nardus*, *Emblica Officinalis*, *Rosmarinus officinalis* and *Simmondsia chinensis* on various biochemical parameters against *P. operculella* for their probable inclusion in the PTM management strategy

Materials and Methods

Rearing Method

The nucleus culture of Potato Tuber Moth was obtained from Entomology, National Chemical Laboratory, Pune. Colony was maintained at 28±2ºC and 60-70% R.H. Adults were maintained in plastic jars covered with black muslin cloth, which is a preferred substrate for oviposition. Adults were provided with 20 % honey solution, while the larvae were provided with pricked potato tubers. Infested potatoes were kept on sterilized soil, which was subsequently used for cocoon formation by larvae. Pupae were kept in separate containers for adult emergence.

Biochemical procedures

For all Biochemical assays 4th instar larvae were used. LC_{20} test oil concentration was used to treat the larvae for 24 hours which were then harvested and homogenized in 20 ml of phosphate buffer of pH 6.8 and centrifuged at 8000 rpm in Ultra Centrifuge (Remi-CM12) for 10 minutes at 4^0C. Ten to twelve larvae weighing about 100 mg were used. The supernatant was used for biochemical assays.

All test oils were dissolved in A.R. grade acetone (200 mg/ml) and serial dilutions were made as per requirement.

A. Amylase activity

Amylase activity was determined by DNSA Method (Plummer 1988, Sumner 1925). The assay mixture containing 0.5 ml substrate (1% Starch), 0.5 ml phosphate buffer (pH 6.8), 0.5 ml enzyme extract was incubated for 10 minutes. 1ml DNSA was added to this mixture. The mixture was kept in boiling water bath for 10 minutes. After this the mixture was cooled, diluted to 10 ml and Absorbance was

recorded at 540 nm on UV spectrophotometer (Systronics-Model 119). Standard curve for glucose was used to calculate the enzyme activity. Each assay was carried out five times and for each set three to five replicates were taken. Data obtained was subjected to statistical analysis.

B. Invertase activity

Invertase activity was determined by DNSA Method (Plummer 1988, Sumner 1925) which was used to detect amylase activity. The only difference was the substrate used was sucrose (5 %) instead of starch. Each assay was carried out five times and for each set three to five replicates were taken. Data obtained was subjected to statistical analysis.

Standard graph for determination of enzyme activity

For standard graph the assay mixture containing 200 µg, 400 µg, 600 µg, 800 µg, 1000 µg, 1200 µg glucose was diluted to 2ml with distilled water. To this mixture 1ml DNSA was added and was kept in boiling water bath for 10 minutes. After this the mixture was cooled, diluted to 10 ml and Absorbance was recorded at 540 nm on UV spectrophotometer (Systronics-Model 119). Graph of glucose concentration versus absorbance was plotted.

C. Estimation of total Carbohydrate contents

Anthrone method was used to estimate carbohydrates. 4 ml of Anthrone reagent was added to 1 ml of a protein free supernatant and was rapidly mixed. This mixture was kept in boiling water bath for 10 minutes. The tubes were then cooled and absorbance was read at 620 nm on UV spectrophotometer (Systronics-Model 119) against the reagent blank. Standard curves for the glucose was used to calculate the amount of carbohydrate. Data obtained was subjected to statistical analysis.

Standard graph for determination of Carbohydrates content

For standard graph the assay mixture containing 200 µg, 400 µg, 600 µg, 800 µg, 1000 µg, 1200 µg glucose was diluted to 2ml with distilled water. To this mixture 4 ml Anthrone reagent was added and was kept in boiling water bath for 10 minutes. After this the mixture was cooled, diluted to 10 ml and absorbance was recorded at 620 nm on UV spectrophotometer (Systronics-Model 119). Graph of glucose concentration versus Absorbance was plotted.

D. Estimation of Total Protein content

Modified Lowry's method was followed for protein estimation (Raghuramulu *et al*. 1983) [16]. Following reagents were prepared for this estimation.

1. Alkaline copper reagent

10 ml of 1% potassium tartarate and 10 ml of 0.5% copper sulphate was added in 20 ml of 10% NaOH with mixing. To this, 50 ml of 20% sodium carbonate was added and mixed thoroughly. The final volume was made upto 100 ml with distilled water.

2. Phenol reagent

1 volume of Folin Ciocalteau reagent (2.0 N) was diluted with 16 volume of distilled water just before use.

To measure protein contents, 0.1 ml of larval extract was taken and volume was raised up to 0.5 ml with Sodium dodecyl sulphate (SDS). 0.5 ml of alkaline copper reagent was added to each tube and was incubated at room temperature for 10 min. After incubation 2 ml of phenol reagent was added to each lube. These tubes were heated at 55°C for 5 min. and were cooled under running water. Absorbance was measured at 650 nm on UV spectrophotometer (Systronics-Model 119). Amount of protein was calculated from standard curve. For each test minimum 10 replicates were taken. The test was repeated eight times. Data obtained was subjected to statistical analysis.

Standard graph for determination of Protein

For standard protein curve bovine serum albumin (BSA, Fraction V) was used. It was dissolved in 1% SDS to give 100 µg/ml concentration. From this, aliquots measuring 20-140 µg were taken for the assay. Volume was raised to 0.5 ml with 1 % SDS. To this, 0.5 ml of alkaline copper reagent was added and mixed well. This mixture was incubated at room temperature for 10 minutes. After incubation 2 ml phenol reagent was added to each tube, and these tubes were heated at 55°C for 5 min. They were cooled under running water and Absorbance was measured at 650 nm. Graph was plotted of actual amount of protein versus absorbance obtained.

Results

Amylase Activity

Table 1 reveals the effects of test oils on amylase activity of Potato tuber Moth. *C. nardus* showed a maximum decrease in amylase activity by 0.50 µmole/ml/min while *R. officinalis* showed a minimum by 0.31 µmole/ml/min. *C. sempervirens* and *E. officinalis* caused decrease in the activity by 0.43 µmole/ml/min and 0.40 µmole/ml/min respectively. The remaining oils, *S. chinensis* and *C. verum* exhibited decrease by 0.36 µmole/ml/min and 0.45 µmole/ml/min respectively in the activity.

Invertase Activity

The effect of test oils on invertase activity of Potato tuber moth are depicted in Table 2. *C. nardus* showed a maximum decrease in invertase activity by 0.50 µmole/ml/min while 0.30 µmole/ml/min was the decrease caused in this activity by *R. officinalis*. The oils of *C. verum* and *E. officinalis* also exhibited decrease in the invertase activity by 0.45 µmole/ml/min and 0.42 µmole/ml/min respectively. A decrease by 0.40 µmole/ml/min and 0.39 µmole/ml/min was exhibited in case of *S. chinensis* and *C. sempervirens* respectively.

Table 1: Effect of the selected plant oils on Amylase Activity.

Name of oil	Amylase Activity (µmole/ml/min)	't'
Control	0.96	-
Cinnamomum verum	0.51	29.60*
Cupressus sempervirens	0.53	12.64**
Cymbopogon nardus	0.46	85.96*
Emblica Officinalis	0.56	17.39*
Rosmarinus officinalis	0.65	35.64*
Simmondsia chinensis	0.60	60.65*

* P< 0.001 ** P< 0.010

Table 2: Effect of the selected plant oils on Invertase Activity.

Name of oil	Invertase Activity (µmole/ml/min)	't'
Control	0.98	-
Cinnamomum verum	0.53	22.3**
Cupressus sempervirens	0.59	39.00*
Cymbopogon nardus	0.48	13.40***
Emblica Officinalis	0.56	72.79*
Rosmarinus officinalis	0.68	25.83**
Simmondsia chinensis	0.58	34.78*

* P< 0.001, ** P< 0.005, *** P< 0.010

Total Carbohydrate Contents

The maximum decrease in the total carbohydrate content (9.50 mg/ gm body weight) was exhibited in case of *C. verum* while *R. officinalis* showed the least decrease (1.71 mg/ gm body weight). *E. officinalis*, *C. sempervirence*, *C. nardus* and *S. chinensis* have exhibited decrease in total carbohydrate content which was accounted in the range of 1.81 mg/gm body weight to 5.51 mg/gm body weight (Table 3).

Total Protein Contents

Table 4 depicts the effects of the selected plant oils on the total protein contents against *P. operculella.* The oil of *C. nardus* decreased the total protein contents by 41.20 mg/gm body weight. The least decrease of 13.20 mg/gm body weight was exhibited in case of *R. officinalis*. The decrease in the protein contents to < 29.60 mg/gm body weight was observed in case of *C. sempervirence* and *C. verum*, while the remaining oils affected the decrease to < 19.6 mg/gm body weight.

Table 3: Effect of the selected plant oils on total Carbohydrate contents.

Name of oil	Total Carbohydrate Contents, (mg/gm body weight)	't'
Control	12.01	-
Cinnamomum verum	02.51	98.51*
Cupressus sempervirens	09.12	7.49*
Cymbopogon nardus	06.54	21.57**
Emblica Officinalis	07.52	83.33**
Rosmarinus officinalis	10.30	10.16***
Simmondsia chinensis	10.20	9.36*

* P< 0.010, ** P< 0.001, *** P< 0.005

Table 4: Effect of the selected plant oils on total Protein contents.

Name of oil	Total Protein contents (mg/gm body weight)	't'
Control	69.6	-
Cinnamomum verum	35.30	84.02**
Cupressus sempervirens	30.40	95.07 **
Cymbopogon nardus	28.40	101.95 **
Emblica Officinalis	44.80	60.15**
Rosmarinus officinalis	56.4	32.05*
Simmondsia chinensis	45.57	59.40 **

* P< 0.010, ** P< 0.001

Discussion

Biochemical moieties in insect play central role in their physiology, development and growth. Therefore, apart from other behavior modifying chemicals, the chemicals affecting the biochemical profile of insects can also play important role in Pest Management Strategies.

Results of the present work revealed that all the test oils have some promise as pest control agents against *P. operculella*. It has reduced amylase, invertase activities and total protein content substantially (0.50 µmole/ml/min, 0.84 µmole/ml/min and 41.20 mg/gm body weight, respectively). Significant reduction in total carbohydrate contents (5.47 mg/gm body weight) is also caused by oil of *C. nardus*

In the remaining oils, oil of *C. sempervirens* exhibited significant reduction in amylase activity (0.43 µmole/ml/min) and total protein contents (39.20 mg/gm body weight).

Significant reduction in total carbohydrate content (4.49 mg/gm body weight), is caused by *E. officinalis*.

Toxic action caused by *C. verum* can be correlated with reduction in enzyme activity, total carbohydrate and total protein contents. While in case of *C. nardus*, delay in development can be correlated with reduction in digestive enzymes as well as total carbohydrates and total protein contents. Delay in development can cause reduction in population (Baker *et al*. 1991).

The effect of the selected plant oils on the biochemical parameters of PTM have certainly opened a new avenue towards the control of the pest with ecofriendly and user friendly method. Field trials of the oils against PTM need to be carried out too.

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