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Evaluation of Insecticidal Activity of Bioactive Compound Isolated from Seeds of *Psoralea corylifolia* L. on Maize Seeds Deteriorating Insect (*Sitophilus zeamais* M.)

Lalitha V.[1]*, Kiran B.[2] and Suresha N.S.[1]

[1]Department of Studies in Botany, Maharanis Science College for Women, JLB Road, Mysore-570005, Karnataka State, India
[2]PG Department of Microbiology, School of Life Sciences, Pooja Bhagavat Memorial Mahajana P.G. Centre, K.R.S. Road, Metagalli, Mysore 570016, India

**Corresponding Author*

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Abstract: Bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one isolated from the seeds of *Psoralea corylifolia* L. were evaluated for insecticidal activity against *Sitophilus zeamais* M. of maize seeds. The seeds were treated with the bioactive compound at 250, 500, 1000, 1500ppm concentration and stored at R.H of 70 ± 5 % for 30, 60, 90 and 120 days. Each bag was then released with 5 pairs of newly emerged adults of *S. zeamais* and adult mortality was noted up to 120 days. After 120 days of incubation, the maize seeds were tested for determination of kernel damage and weight/volume ratio, total protein content, carbohydrates, total uric acid and total thiamine.

Keywords: *Psoralea corylifolia*, *Sitophilus zeamais,* Mortality, Maize seeds, Kernel damage, Protein, Carbohydrates, Uric acid

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Introduction

In agriculture, post harvest losses of food grain are important factor in the world food supply. The losses are primarily due to infestation by insects, microorganisms and also due to rodents and birds. A smaller but quite significant proportion of the total losses results from gradual deterioration of viability, nutritive quality and end use properties during storage under commercial conditions. Few insects viz., weevils, beetles, and moths infest undamaged grains and are often found in the field before harvest. These insects invade grains that are already damaged or weakened. They can include mites, ants, and other scavengers. Insects can damage grains by feeding on them, leading to

loss of weight and nutritional value. They may introduce moulds and fungi, further deteriorating the quality of stored grains. Some insects produce toxins that can contaminate the grains and pose health risks. For millions of people, food supplies depend almost entirely on each year's harvest. Protecting our harvested crops from insects pests can significantly increase available food resources because at least 10% of our harvested foods are destroyed in storage by insects pests. Losses of 30% are common throughout large areas of the world (Hall, 1970). Prevention of biodeterioration of grains is generally achieved by treatments with synthetic fungicides used in the prevention of biodeterioration of grains which are also known to be toxic to non-target organisms and cause side effects to man (Mordue *et al*., 1998). Excessive use of these chemical poisons has resulted in environmental issues, including the negative impact on non-target species in the natural agricultural environment, disruption of the ecological balance between pests and predators, pesticide resistance in target species, health risks, and air pollution (Hyder *et al*., 2024). Exploitation of bioorganic molecules for the prevention of biodeterioration of grains would help to overcome these ill effects. Medicinal plants are useful in the treatment of many ailments and diseases among rural dwellers, indigenous users, traditional medicine (TM) practitioners, and livestock owners in many African countries (Christiana *et al*., 2021). Considering these, a systematic investigation was envisaged to evaluate the potency of 2H-Furo[2,3-H]-1-benzopyran-2-one compound isolated from *P. corylifolia* L. belongs to family Fabaceae for prevention of biodeterioration of maize grains during storage.

Materials and Methods

Collection of seed sample and determination of moisture:

Maize seed samples (20 kg) was directly collected from seed market, Mysore, India and brought to the laboratory for further experiments. The sample was subjected to determination of moisture content (%) by following the procedures of high constant temperature oven method (Mordue *et al*., 1998).

Plant material:

Fresh and healthy seeds of *P. corylifolia* L., were washed with tap water thrice and two to three times with distilled water. The seeds were air dried at room temperature. Completely air dried seeds were powered using waring blender (Waring International, New Hart-Ford, CT, USA).

Isolation of the Bioactive compound:

25 g of the powdered seeds of *P. corylifolia* were extracted with petroleum ether and methanol mixture in the ratio 9:1 (v/v) by refluxing for 8 h at 50- 60°C in a Soxhlet apparatus. The excess of solvent was removed by distillation under reduced pressure. The concentrated extract was cooled for 48 h at 5°C to obtain pure compound as crystals. The crystals was dissolved in chloroform and eluted by silica gel (6x120 mesh size) column chromatography (2x40 cms) using n-hexane: chloroform (9:1)(v/v). The purity of the bioactive compound was confirmed by TLC and R_f value was determined .

Structural elucidation of the Bioactive compound:

IR (Infra red) analysis: IR spectra were recorded in KBr pellete on a Perkin-Elmer FTIR 1650 spectrometer (Al-Fatimi *et al*., 2006).

^{1}H- NMR data analysis: The ^{1}H- NMR spectrum of the bioactive compound, 2H-Furo [2,3-H]-1-benzopyran-2-one was recorded on a Bruker AM 400 F (400 MHZ) NMR spectrometer using $CDCI_3$ as a solvent and TMS as internal standard. All chemical shift values were expressed in δ scale as s= singlet, d= doublet, t= triplet, m= multiplet (Al-Fatimi *et al*., 2006).

^{13}C NMR analysis: The ^{13}C-NMR spectra were obtained on a Burker spectrometer AM 400 (400 MHZ) with the solvent signal as internal reference (Al-Fatimi *et al*., 2006).

Gas Chromatography-Mass Spectroscopy (GC- MS) analysis: Mass spectrum of compound was recorded on MS data review active chromatogram

with range 40-600M/Z (Yanez *et al*., 2005). The other characterizations of the data of the compound were also determined.

Antifeedent activity:

Effect of Bioactive compound [*2H-Furo*[*2,3-H*]*-1-benzopyran-2-one*] *on mortality of Sitophilus zeamais*:

Insect culture: Standard laboratory cultures of *Sitophilus zeamais* were collected from Food Protectants and Infestation Control Department, CFTRI, Mysore. The cultures were maintained in our laboratory in maize seeds at 30±1° C and 70±5% RH. Plastic containers covered with muslin cloth served as rearing cages.

Bioassay: Maize seeds (100 g in 5 replicates) were sterilized at 45°C for 8 h in order to kill the eggs and developing larvae (if any). The seeds treated with the bioactive compound isolated from the seeds of *P. corylifolia* at 250, 500, 1000 and 1500 ppm concentration were taken in a polyethylene bag and covered with muslin cloth. All the bags were shaken thoroughly for uniform distribution of the bioactive compound. Seeds without the bioactive compound treatment served as control. Each bag was then released with 5 pairs of newly emerged adults of *S. zeamais.* Treated and control seeds were incubated at room temperature (30±1°C) at R.H. of 70 ± 5 % for 30, 60, 90 and 120 days. Observations on the adult mortality were made up to 120 days (Bright *et al*., 2001). The per cent mortality was calculated using Abbott's formula (Abbott's, 1925):

$$\text{Corrected mortality} = 100x \frac{\text{\% alive control - \% alive treated}}{\text{\% alive control}}$$

Effect of the bioactive compound [*2H-Furo*[*2,3-H*]*-1-benzopyran-2-one*] *on nutritional quality of maize during storage*:

Determination of moisture content: The treated and untreated seed samples were subjected to moisture content (%) analysis by following the procedures of high constant temperature oven method (Satish *et al*., 1999).

Determination of kernel damage and weight/ volume ratio: Seed samples were drawn at 30, 60, 90 and 120 days and % kernel damage and loss of weight due to insect infestation was determined by comparing with control (Rajan *et al.*, 1975).

Determination of total protein content: Extraction is usually carried out with buffers used for the enzyme assay. 500 mg of the sample was weighed and grind well with a pestle and mortar in 5-10 ml of the buffer, centrifuged and supernatant was used for the protein estimation. 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard was pipetted out into a series of the test tubes. 0.1 ml and 0.2 ml of the sample extract was pipetted out in two other test tubes. The volume was made up to 1 ml in all the test tubes, a tube with 1 ml of water served as the blank. 5 ml of reagent C was added to each tube including the blank, mixed well and allowed to stand for 10 min. Then 0.5 ml of reagent D added, mixed well and incubated at room temperature in the dark for 30 min, blue colour was developed. The readings were taken by spectrophotometer at 660 nm. A graph was drawn and the amount of protein in the sample was calculated and expressed in g/100 g of seeds sample (Lowry *et al.*,, 1951).

Determination of carbohydrates: 100 mg of the sample was weighed into a boiling tube. Hydrolyzed by keeping it in a boiling water bath for 3 h with 5 ml of 2.5 N HCl and cooled to room temperature. 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard was pipetted out into a series of test tubes. 0.1 and 0.2 ml of the sample solution was pipetted out in two separate test tubes, the volume was made up in each tube to 1 ml with water. A blank was set with 1 ml of water. 1 ml of phenol solution was added to each tube. 5 ml of 96% Sulphuric acid was added to each tube and shaken well. After 10 min the contents were shaken in the tubes and placed in a water bath at 25-30°C for 20 min. The reading of the colour was taken at 490 nm by spectrophotometer. Total amount of carbohydrates present in the sample was calculated using standard graph (Dubois, 1956).

Determination of total uric acid: 4 g sample (5

replicates) was mixed with 5 ml glutathione solution (10 mg/ml) and left overnight. To this mixture, 25 ml 1 N sodium hydroxide (pH 9.0-9.3) was added and transferred to a 100 ml glass stoppered cylinder. The solution was made up to 100 ml with sodium acetate solution (5%) and then mixed, the mixing being continued every 10 min for 1 h. From this, 15 ml of the solution was taken and centrifuged for 30 min at 3000 rpm. Supernatant (4 ml) of each sample was taken into 2 test tubes (marked 1 and 2) and 1 ml of borate buffer (0.01 M, pH 9.2) was added and mixed well. To the 3rd test tube and test tube No.1, 5 ml of uricase solution (100 mg in 50 ml of 0.01 M sodium borate buffer) was added and mixed well. Then, the contents of tube No. 2 and 3 were mixed and the absorbance was read immediately by spectrophotometer at 292 nm against solution in test tube No. 1 as blank. Similarly control samples of each product were also carried out as earlier and their OD was subtracted from their respective infested samples, as the absorbance values. The uric acid content (mg/100 g) was calculated using standard graph. Uric acid standard solution (containing 100 µg per ml 5% sodium acetate) corresponding to 0-10 µg uric acid per ml in the final solution was taken and the procedure described above was followed. From the absorbance value a standard curve was drawn (Wolf *et al*., 2007).

Determination of total thiamine: 5 g of finely ground sample was weighed into a 250 ml conical flask in duplicate. One hundred ml of 0.1 N H_2SO_4 was added slowly without shaking. The flask was stoppered and allowed to stand overnight. Next morning it was shaken vigorously and filtered through Whatman No.1 filter paper, discarding the first 10 to 15 ml of filtrate. 10 ml of the extract was pipetted out in duplicate into 100 ml separating funnel. 10ml of working standard solution was pipetted (in 4-5 replicates). 3 ml of 15% NaOH was added into each separating funnel immediately followed by four drops (0.2 ml) of ferricyanide solution. Then it was shaken gently for exactly 30 sec. 15 ml of isobuthanol was added rapidly from a quick delivery burette or to a measuring cylinder. Stopper immediately, mixed vigorously for 60 sec and allowed the layers to separate. The bottom layer was drained carefully and discarded. One spatula full of sodium sulphate was added directly into the separating funnel, stoppered and swirled gently to clarify the extract. If the extract was not clear, little more Na_2SO_4 was added to clarify. The clear extract was collected from the top using a Pasteur pipette into a clean dry test tube. A set of sample blanks was prepared by pipetting out 10 ml of the extract and a blank for the standard was prepared separately. Suitable primary (366 nm) and secondary filters were selected as per the make of the fluorimeter. Fluorimeter was set by initially adjusting the standard blank to 0 reading and standard to 100. Then sample blank and sample was read (Sadasivam and Manickam, 1996).

Results and Discussion

Collection of seed sample and determination of moisture content: The moisture content of the seed sample was 13.5%.

Structural elucidation of the Bioactive compound:

IR (*Infra red*) *analysis*: The IR spectrum of 2H-Furo[2,3-H]-1-benzopyran-2-one showed absorption band in the region of 1652.9 cm^{-1} for-C-O- stretching. Further absorption bands at 1550.7cm^{-1} and 1454cm^{-1} were due to the presence of coumarin ring oxygen and furan ring oxygen, respectively. The IR spectra of the bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one is shown in Figure 1.

^{1}H- NMR data analysis: In ^{1}H- NMR spectra, the signal due to C_3-H and C_4-H of coumarin appeared at δ 6.41 as doublet and at δ 7.81 as singlet. The aromatic protons (C_7 + C_8) are mingled together and appeared at δ 7.7 as multiplet. The signal due to the $C_{3'}$ and $C_{2'}$ protons appeared at 6.84 and 7.49 as doublet, respectively. The NMR spectra of the bioactive compound, 2H-Furo[2,3-H]-1-benzopyran-2-one is shown in Figure 2.

^{13}C NMR analysis: 13C- NMR data of the bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one are recorded as solvent $CDCl_3$, δ146.85(C_2),

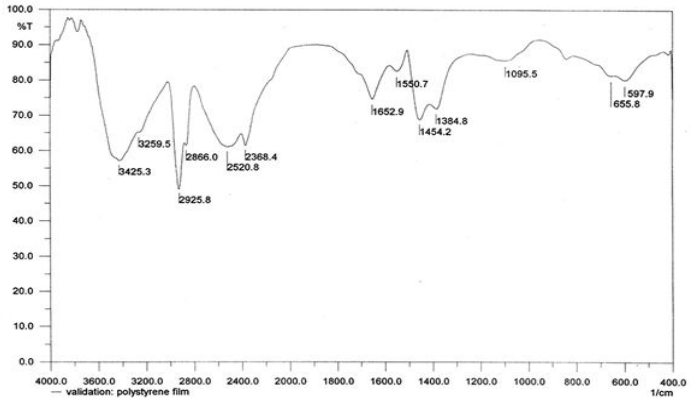

Fig. 1: IR(Infra red) spectra of the bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one, isolated form the seeds of *P. corylifolia.*

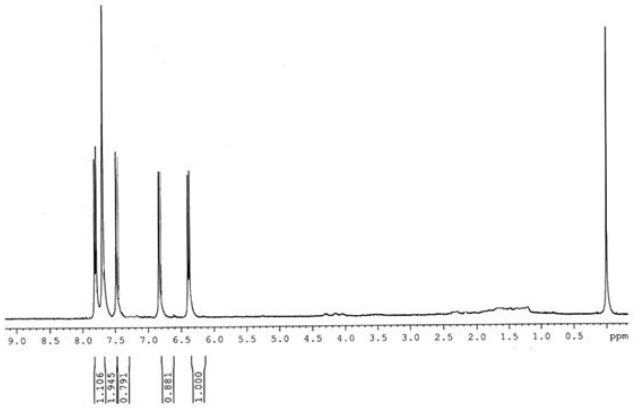

Fig. 2: ^{1}H-NMR spectra of the bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one, isolated form the seeds of *P. corylifolia.*

δ143.8 (C_4), δ119.77(C_3), δ115.5(C_7), δ114.76(C_8), δ_2 106.3($C_{3'}$) and δ 99.86 ($C_{2'}$) atom (Fig. 3).

Gas Chromatography - Mass Spectroscopy (*GC- MS*) *analysis*: Showed the molecular ion peak at M/Z 186.17 consistent of molecular formula $C_{11}H_6O_3$. The peak at M/Z 158 was due to the formation of coumarin cation. The recorded chromatogram of plot matches with chromatogram of known compound 2H-Furo[2,3-H]-1-benzopyran-2-one (Fig. 4).

Figure 5 presents the molecular structure of the bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one.

Antifeedent activity:

Effect of Bioactive compound [*2H-Furo* [*2,3-H*]*-1-benzopyran-2-one*] *on mortality of Sitophilus zeamais:* It was observed that in the untreated control seeds, the number of storage insects *S. zeamais* increase significantly with increasing the period of storage. At 0 days of storage, the number of insects was 10 and after 30 days there was a slight increase in number and it was 13; after 60 days, the insects number was recorded 35 and after 90 and 120 days of storage the number of population was highly increased to 103 and 185, respectively. In the seeds treated with 250 ppm concentration of the bioactive compound, it was also observed that with increasing period of storage there was increase in the number of adult *S. zeamais.* At 0 days, the number of population recorded was 10 and at 30 days of storage there was a marginal decrease in number of *S. zeamais*. But after 60 days of storage, marginal increase

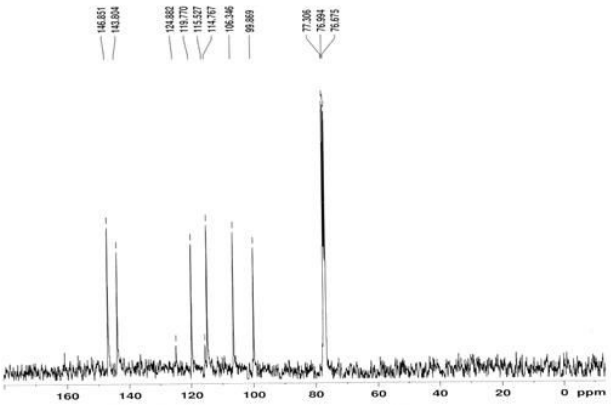

Fig. 3: ^{13}C-NMR spectra of the bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one, isolated form the seeds of *P. corylifolia.*

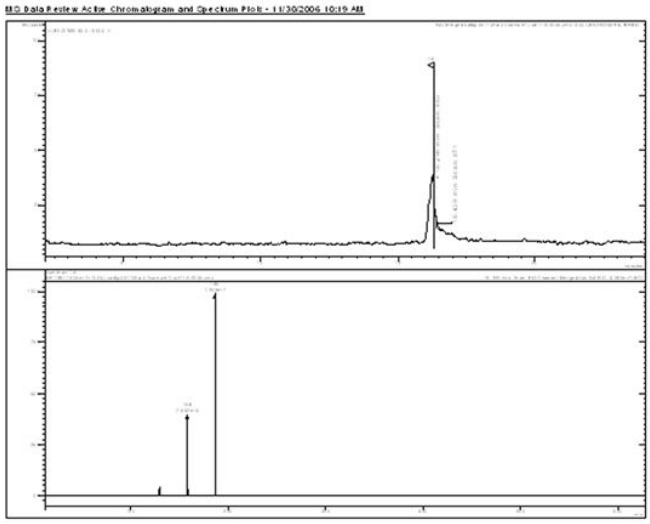

Fig. 4: Gas Chromatograph-Mass Spectrum(GC-MS) spectra of the bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one, isolated form the seeds of *P. corylifolia.*

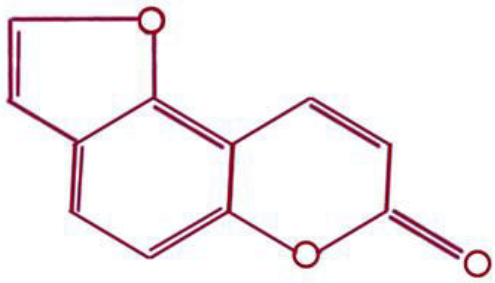

Fig. 5: Molecular Structure of the bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one, isolated form the seeds of *P. corylifolia.*

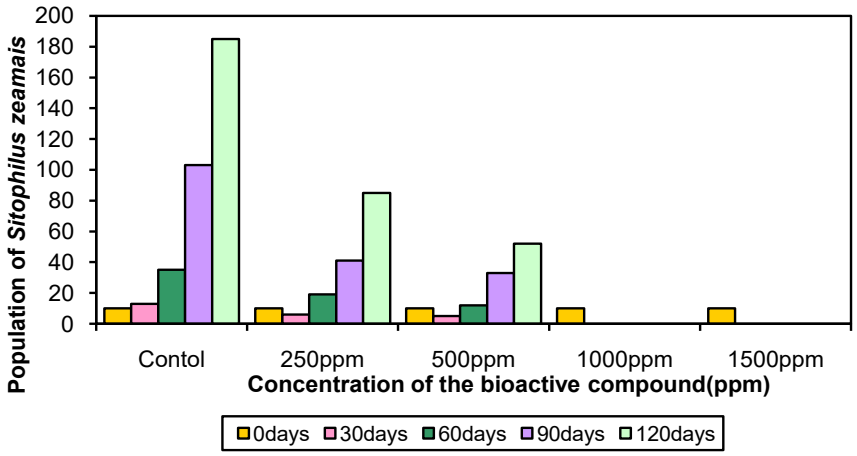

Fig. 6 : Effect of the bioactive compound [2H-Furo [2,3-H]-1-benzopyran-2-one] of seeds of *P. corylifolia* L. on mortality of *Sithophilus zeamais.*

was recorded (19). However, after 90 days of storage there was a highly significant increase in their number. which further increased to 85 after 120 days of storage. At 500 ppm concentration treatment the number of *S. zeamais* was 10 at 0 days and at 30 days of storage, the number of population slightly decreased to 5. After 60 days of storage there was a marginal increase (12) and after 90 days of storage a gradual increase in number was recorded. In the seeds treated with 1000 and 1500 ppm concentration of the bioactive compound the number of insects was 10 at 0 days and all insects were killed after 30 days of storage. Thus there were no live insects in both the treatments (Fig. 6).

Effect of the bioactive compound [*2H-Furo*[*2,3-H*]-*1-benzopyran-2-one*] *on nutritional quality of maize during storage*:

Determination of moisture: The moisture content of seeds treated with different concentrations of the bioactive compound stored for different period and the untreated seeds is presented in Table 1. The moisture content of the untreated control seeds at 0 days was 13.5% and it increased gradually with increasing the period of storage. In the seeds treated with 250 ppm concentration of the bioactive compound, there was a marginal increase in moisture content. In the seeds treated with 500 ppm concentration of the bioactive compound, a marginal increase in moisture content was observed at 0 days, it was 13.5% and after 120 days of storage it was 14.5%. In the seeds treated with 1000 and 1500 ppm concentration of the bioactive compound, there was no change in moisture content (Table 1).

Determination of protein: In case of seeds treated with 500 ppm concentration of the bioactive compound, slight decrease in protein content was observed. After 120 days of storage, the protein content was 10.6 g/100 g of seeds. In case of seeds treated with 1000 and 1500 ppm concentration of the bioactive compound, the protein content which was 11.1 g at 0 days of storage remained unchanged even after 120 days of storage (Table 1).

Determination of carbohydrate: The carbohydrate content of untreated and seeds treated with different concentrations of the bioactive compound [2H-Furo[2,3-H]-1-benzopyran-2-one] stored for different periods is presented in Table 1. In case of seeds treated with 500 ppm concentration of the bioactive compound, at 0 days of storage, the carbohydrate content was 66.2 g/100 g of seeds. In case of seeds treated with 500 ppm concentration of the bioactive compound, at 0 days of storage, the carbohydrate content was 66.2 g/100 g of seeds. With increasing the period of storage, the carbohydrate content

Table 1: Effect of bioactive compound [2H-Furo[2,3-H]-1-benzopyran-2-one] on nutritional quality of maize seeds at different duration of storage period-*Sitophilus zeamais*

Concentration	Periods	Moisture (%)	*Protein* (g)/100 g	Carbohydrate (g)/100 g	Kernel damage (%)	Uric acid mg/100 g	Thiamine mg/100 g
Control	0	13.5	11.1	66.2	0.0	0.0	0.90
	30	13.8	10.8	66.0	15	17.0	0.85
	60	14.0	10.2	63.2	41	35.6	0.76
	90	14.5	9.7	61.1	87	81.0	0.65
	120	15.7	9.0	60.0	96	99.0	0.52
250 ppm	0	13.5	11.1	66.2	0.0	0.0	0.90
	30	13.6	11.0	66.0	9	12.0	0.88
	60	13.9	10.8	65.9	31	25.3	0.82
	90	14.0	10.6	64.5	58	45.9	0.79
	120	14.3	10.1	63.1	69	63.5	0.72
500 ppm	0	13.5	11.1	66.2	0.0	0.0	0.90
	30	13.7	11.0	66.0	5	5.0	0.90
	60	13.8	10.9	65.8	19	12.6	0.89
	90	13.9	10.8	65.5	30	22.8	0.85
	120	14.5	10.6	65.0	45	39.6	0.82
1000 ppm	0	13.5	11.1	66.2	0.0	0.0	0.90
	30	13.5	11.1	66.2	0.0	0.0	0.90
	60	13.5	11.1	66.2	0.0	0.0	0.90
	90	13.5	11.1	66.2	0.0	0.0	0.90
	120	13.5	11.1	66.2	0.0	0.0	0.90
1500 ppm	0	13.5	11.1	66.2	0.0	0.0	0.90
	30	13.5	11.1	66.2	0.0	0.0	0.90
	60	13.5	11.1	66.2	0.0	0.0	0.90
	90	13.5	11.1	66.2	0.0	0.0	0.90
	120	13.5	11.1	66.2	0.0	0.0	0.90

showed marginal decrease and it was 65 g/100 g of seeds after 120 days. In case of seeds treated with 1000ppm and 1500ppm concentrations of the bioactive compound, it was 66.2 g/100 g of seeds at 0 days of storage (Table 1).

Determination of kernel damage: After 90 and 120 days of storage, highly significant kernel damage was recorded which was 18% and 96%, respectively. In the seeds treated with 250 ppm concentration of the bioactive compound, at 0 and 30 days of storage period, it was 0 and 9% kernel damage, respectively. In case of seeds treated with 500 ppm concentration of the bioactive

compound, significant decrease in kernel damage was observed. In case of seeds treated with 1000 ppm and 1500 ppm concentration of the bioactive compound, at 0 days of storage, it was 0.0% and with the increasing the duration of storage no increase in kernel damage was observed even after 120 days of storage (Table 1).

Determination of uric acid: In case of seeds treated with 250 ppm concentration of the bioactive compound at 0 days of storage it was 0 mg/100 g of seeds. After 30, 60, 90 and 120 days storage it was 12, 25.3, 45.9 and 63.5 mg/100 g of seeds, respectively. In case of seeds treated with 1000 and 1500 ppm concentrations of the bioactive compound it was 0 mg/100 g of seeds at 0 days of storage and with increasing the periods of storage the uric acid content was remained the same even after 120 days of storage (Table 1).

Determination of thiamine: In case of seeds treated with 250 ppm concentration of the bioactive compound at 0 days it was 0.90 mg/100 g of seeds. After 120 days of storage the marginal decrease in thiamine content (0.82 mg/100 g of seeds) was noticed. In case of seeds treated with 1000 and 1500 ppm concentration of the bioactive compound, at 0 days of storage it was 0.90 mg/100 g of seeds. In both the treatments with increasing period of storage up to 120 days the thiamine content remained the same (0.90 mg/ 100 g of seeds) (Table 1).

The results of the experiments conducted with regard to the effect of treatment of maize grains with different concentrations of the bioactive compound isolated from seeds of *P. corylifolia* on the mortality of *S. zeamais* revealed that during storage it is encouraging. A highly significant increase in the *S. zeamais* population was observed in the untreated control seeds with increasing periods of storage. A marginal increase in their population was observed in 250 ppm and 500 ppm concentration treatment, where as total mortality of all the test insects were observed at 1000 and 1500 ppm concentration at and beyond 30 days of storage. Thus the results of the present investigation suggests that at and beyond 1000 ppm concentration is lethal to these insects. Experiments conducted in the present investigation with regard to other quality parameters like moisture content, protein content, carbohydrate content, uric acid content, thiamine content and kernel damage in maize seeds treated with different concentrations of the bioactive compound also revealed that 1000 ppm and 1500 ppm concentration treatment does not affect any of these quality parameters. None of the earlier workers have demonstrated the efficacy of this bioactive compound in the management of storage insects in maize grains.

Conclusion

It is evident from the results of the present investigation that treatment of maize grains with 1000 ppm concentration of the bioactive compound, 2H-Furo [2,3-H]-1-benzopyran-2-one isolated from seeds of *P. corylifolia* could be exploited for the prevention of moulding, mycotoxin contamination and biodeterioration and destruction of seeds by insects of maize during storage. However, further investigations are necessary with regard to toxicological aspects of this compound before it is finally recommended for commercial exploitation.

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