Indian J. Applied & Pure Bio. Special Volume 89-99 (2021).
Environment & Sustainable Development : Problems, Prospects & Mitigation Date : 25th-27th November, 2021
ISSN: 0970-2091
A web of Science Journal

Insecticidal activity of the seed extracts of *Annona squamosa* Linn. against *Callosobruchus analis* in green gram, *Vigna radiata* Linn. R. Wilczek

S. Nandhini, G. Maheswari and M. S. Santhanambika*

*Research Scholar, Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore-641011 (India)
Mail Id: santhanamabikasakthivel096@gmail.com

Abstract

Stored grain pests are one of the major constraints in affecting the quantity and nutritional quality of the food grains in storage areas. An attempt was made in the laboratory to test the efficiency of indigenous *Annona* seed extracts to manage pulse beetles, *Callosobruchus analis*.The test insect, *Callosobruchus analis*, was collected from the pulses Department of Tamil Nadu Agricultural University, Coimbatore. Green grams were procured to culture the insects and maintained at a BOD incubator at 28 ± 2°C and 70-75% relative humidity. Newly emerged adults within 24 hours of emergence were used for bioassay studies.The seeds of *Annona* (*Annona squamosa*) collected from the local fruit stalls were dried and pulverized.The seed powders were subjected to Soxhlet extraction using solvents such as petroleum benzene, chloroform, acetone and alcohol based onthe order of their increasing polarity.The test solutions of different concentration (1%, 3%, & 5% doses) were prepared and mixed with 25g of green gram. Five pairs of test insects were introduced, three replications were kept for each concentration and controls.Observations were made on the adult mortality, adult emergence, weight loss of seeds, germination and vigor index of seedlings.The result on the mean values of mortality of *C.analis* in *Annona* treated green gram showed cent percent mortality on the first day itself at higher concentration levels(1%, 3%, 5%) moderate variation of mortality was noticed between control and the treatments of lower concentration (1000, 5000, 10000 ppm).

Providing an adequate supply of food and improving the health of a rapidly increasing human population are the two major challenges of our country. Foodgrain production in India is enough to feed its population if storage losses are minimized. A major cause of substantial storage losses is the lack of awareness about safe storage and pest management practices.Pulses are of great importance in the human diet as they are rich in nutrition, particularly proteins, micronutrients, minerals, vitamins, and dietary fibers. Along

with the cereals, they also form a part of the staple food for Indians[9]. One such vital pulse native to India is green gram *Vigna radiata* Vilczek, it is very rich in protein with higher digestibility and could be served to convalescing babies or people with malnutrition[8]. Its high protein content and the moist condition of storage areas pave the way for insect infestation especially the pulse beetle, *Callosobruchus analis* belonging to the family of Chrysomelidae under the order Coleoptera. It is essential to monitor the occurrence of these pests as it contaminates the food commodity leading to economic losses for the farmers and traders. Various medicinal plantshave been employed in the prevention and treatment of different health ailments from time immemorial. The pharmacological activities of these plants are due to the presence of secondary metabolites and these are now investigated extensively in the plants as a source of medicinal agents[7,11,15]. *Annona*ceae, the custard apple family with about 2500 species and more than 130 genera. This family has flowering plants, trees, shrubs[10]. Of all, custard apple, *Annona squamosa,* has been widely used as a natural medicine in the tropic, including the bark, leaves, root, fruit, and seeds. Different properties and uses are attributed to thedifferent parts of the tree[3]. The present investigation aims to evaluate the insecticidal potency of the seed extracts of *Annona squamosa* against the pulse beetle, *Callosobruchus analis.*

An investigation was carried out in the laboratory to test the efficacy of *Annona* seed extract against the pulse beetle, *Callosobruchus analis* in green gram, *Vigna radiata* Wilczek.

Preparation of green gram :

Green gram was procured from the local market, cleaned manually to remove infested seeds and debris then sun-dried for 2 hours. The dried seeds were transferred to clean glass jars of 1000ml capacity and closed with perforated lids for good aeration. It is kept at (28±30°C Temperature, 70±80% Relative Humidity) for about three weeks to remove all prior infestation by the pest before bioassay studies.

Collection and maintenance of Callosobruchus analis culture

The insect used in the present study was obtained from the Department of Pulses, Tamil Nadu Agricultural University (TNAU), Coimbatore.A healthy culture was maintained in the BOD incubator at 28 $\pm$ 2°C and 70-75% RH in clean glass jars of 500ml capacity containing green gram. The glass jars were covered with perforated lids for good aeration. Adults that emerged from the culture within 24 hours were used for the bioassay studies.

Preparation of seed extracts :

Fresh seeds of *Annona* fruit were collected, dried under laboratory conditions, pulverized and packed in polythene bags until required. 10g of seed powder was subjected to Soxhlet extraction using Petroleum benzene, chloroform, acetone, and alcohol as solvents based on increasing order of polarity. The leaf extracts were distilled, and the residues were kept in a water bath below 40°C for solvent evaporation. The residue obtained was stored for future use.

Bioassay Studies :

A laboratory experiment was laid in a

Completely Randomized Design (CRD). 25g of green gram was taken in small polythene bags and mixed with 5 ml of different concentrations of each seed extract. The untreated and acetone-treated green gramswere maintained as controls. All the treatments and controls were replicated three times. In each replicate, five pairs of newly emerged adults were released after the evaporation of solvents. The polythene bags were tightly closed. The various parameters studied to determine the efficiency of seed extracts included adult mortality, adult emergence, weight loss in grains, seed germination, and vigor index.

Percentage mortality of Green gram :

Observations on the mortality of insects were recorded for each treatment and control at every exposure time. The dead and moribund insects were taken as dead and removed from grain at the time of counting. Percentage mortality was calculated using the following formula

Dead insects
Percentage mortality of insects =------ X 100
Insects introduced

Adult emergence

F_1 adult emergence of green gram was recorded on the 30th Day After Treatment (DAT). The emerged insects were separated from the seed using a grain sieve (2 mm diameter).

Percentage weight loss :

To calculate the weight loss of the green gram, control and treated grains were sieved to remove all the dust and insects and weighed using an electronic balance. The percent weight loss was calculated by the formula[5].

$$\text{Percentage weight loss} = \frac{\text{Original Weight} - \text{Current Weight}}{\text{Original Weight}} \times 100$$

Germination Percentage and vigor index :

For evaluating the impact of seed extracts on seedling growth, the seeds of 30 DAT (Days After Treatment) were germinated in germination towels following the wet towel method. Five seeds from every treatment and control were taken and soaked in distilled water for 3 hours. The seeds after decanting the water were arranged in germination towels and rolled After one week, the numbers of germinating seeds of green gram were counted and the seedling parameters such as root length and shoot length were also measured. The vigor index of the seedling was calculated using the following formula.

Vigor index = Germination percentage X Mean length of root + Mean length of shoot

Phytochemical Screening :

Phytochemical analysis of the seed extracts was carried out simultaneously for different solvents by following standard procedures[12,14]. Tannins, saponins, reducing sugars, alkaloids, terpenoids, flavonoids, cardiac glycosides and anthraquinones were estimated following standard methods.

Qualitative Analysis:

Tannins

The extract was(0.5g) dissolved in 10 ml of distilled water, then a few drops of 1%

ferric chloride solution was added to obtain a brownish-green or blue-black precipitate, which confirms the presence of tannin.

Saponins

The extract was(0.5g) dissolved in 5 ml distilled water. The mixture was shakenvigorously. The formation of stable, persistent froth shows the presence of saponins. Further addition of 6 drops of olive oil while shaking forms an emulsion, confirming the presence of saponins.

Alkaloids

The extract was(6ml) mixed with 6 ml of 1% HCl in the steam bath, then it was filtered. 1 ml of Mayer's reagent was added. The presence of turbidity shows the presence of alkaloids. Further addition of a few drops of olive oil to form an emulsion confirmed the presence of alkaloids.

Terpenoids

The extract was(0.5g) dissolved in 2 ml of chloroform, then 3 ml concentrated sulfuric acid was added. A reddish-browncolor in interphase indicates the presence of terpenoids.

Flavonoids

Five ml dilute ammonia was added to 5 ml extract, and then 5 ml concentrated sulfuric acid was added. The formation of yellow color shows the presence of flavonoids.

Cardiac glycosides

The extract was (2.5g) added to 2.5 ml of distilled water. 1 ml glacial acetic acid containing a few drops of ferric chloride was added, followed by 0.5 ml of concentrated sulfuric acid. The presence of a brown ring at the interphase indicates the presence of deoxy sugar. A violet ring below the brown ring was observed, while a greenish ring also appears above the brown one, confirming Cardiac glycosides' presence.

Anthraquinones

The extract was (2.5) dissolved in 5 ml of concentrated sulfuric acid and filtered. The filtrate was dissolved in 2.5 ml of chloroform. The chloroform layer was pipetted into a tube and 0.5 ml of 10% diluted ammonia was added. The formation of pink-red or violet color shows the presence of anthraquinones.

Phenol

The extract was (2ml) dissolved in 4 ml of distilled water and added few drops of 10% $FeCl_3$. The appearance of blue or green color indicates the presence of phenols.

Detection of carbohydrates :

Molisch's test: To two ml of filtrate, two drops of alcoholic solution of α naphthol was added and the mixture was shaken well. 1ml of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand. Formation of violet ring indicates the presence of carbohydrates.

Fehling's test: One ml of the filtrate was boiled in a water bath and 1ml each of Fehling's solution A and B was added to it. Brick red precipitate was formed, indicating the presence of sugar.

Detection of protein and amino acids:

Millon's test: To two ml of filtrate,

few drops of Millon's reagent were added. A white precipitate indicates the presence of proteins.

Biuret test: Two ml of filtrate was treated with one drop of 2% copper sulphate solution. To this, 1ml of ethanol (95%) was added, followed by 2-3 potassium hydroxide pellets. Pink color in the ethanol layer indicates the presence of proteins.

Ninhydrin test: Two drops of ninhydrin solution was added to 2 ml of aqueous filtrate. A characteristic purple color indicated the presence of amino acids.

2.6. Statistical analysis :

The data on percentage adult mortality and percentage weight loss was transformed into ARC sign values before being subjected to ANOVA. A two-way Analysis of Variance was used to analyze the collected data. The mean difference of each variable was further analyzed using Duncan's Multiple Range Test (DMRT).

Impact of Annona seed extract on the mortality percentage of C.analis :

The mortality percentage of the seed extracts are given in table number 1. Significant variation was noticed between the control and different treatment. Due to moderate mortality variation between control and different treatment, the dosage level of each extract has been increased from ppm (parts per million) to percentage level. Therefore, the dosages selected for the present study included lower concentrations of 1000, 5000 and10,000 ppm and higher concentrations of 1%, 3% and 5%. However, the mortality percentage recorded for higher concentrations of 1%, 3% & 5% were 100% on the 1st Day After Treatment (DAT). Therefore, the results pertaining to the lower concentration were tabulated.

Analysis of the mean values of the mortality on different days indicated that increase in mortality percentage from 1st day - 4th Day After Treatment(13.71% – 26.39%) and the percentage declined from 4th day onwards up to 8th day (26.39%-2.93%). Therefore,the peak mortality range was obtained on the 4th Day After Treatment (26.39%).

The highest mortality of 30.00% was recorded for 10,000ppm Petroleum benzene while the control and acetone treated control recorded no mortality on 1st day of observation. Chloroform extract of 1000 and 5000ppm concentration levels registered the highest mortality on 2nd Day After Treatment (26.67%). However, the control recorded no mortality on this day.Similar observation recorded seed extract of *Annona* at 1.5 per cent concentration produced highest mortality in *H. armigera* (43.33%) and 36.66 per cent mortality at 1% concentration in *S. litura*[13]. Natural death of insects was observed in control from 3rd day onwards (10.00%) and all the treatment recorded the mortality percentage ranging from 10.00%-26.67%. From the 4th day onwards, the mortality percentage of control and Acetone control (18.43%-33.21%) recorded the maximum death compared to other treatments (6.14 - 30.99%).The mean mortality value attained peak on this day of treatment (20.71%). From the 5th day onwards, the mortality

percentage of treatment either attained cent percent level (10,000 dose of Petroleum benzene, Alcohol and Chloroform) or minimum mortality (3.33-20.00%). Impact of *Annona* seed extract on *C.analis* on 6th, 7th and 8th Day After Treatment recorded a gradual decrease in mean mortality percentage from 8.81%, 9.28% and 2.14, respectively.

On the 1st Day of observation of mortality in our study, 10,000 ppm petroleum ether extract of *Annona* recorded higher percentage of mortality (30%) This is in accordance with Petroleum ether leaf extracted from leaves of *A. squamosa* were reported to possess an insecticidal and growth regulating activities on three mosquito species namely *Anopheles stephensi, Culex. quinquefasciatus* and *Aedes aegypti*[6].

Among the solvents tested for its efficacy in our stydy, acetone extract recorded minimum adult emergence and percentage weight loss. The acetone extracts from fresh and stored leaves of *Annona* were toxic to adult of *C. maculatus*, whereas the ethanol extracts were not active[2].

Overall data confirmed that the solvents used for extraction of *Annona* seed (Petroleum benzene,Chloroform, Acetone and Alcohol) were effective in bringing out the cent percent mortality on the 1st day itself at higher concentration levels (1%,3%, and 5%). Moderate mortality variation was noticed between control; acetone-treated control and the treatments of the lower concentration (1000, 5000 and 10,000ppm).

Impact of Annona seed extract on the number of adult emergence in Green Gram:

Analysis of the data on the number of adult emergence in the treatment and control showed the significant variation among them. Dose-dependent variation of adult emergence was noticed for chloroform, acetone and alcohol extract.

The adult emergence of *Annona* seed extract at 1%, 3% and 5% concentration of all the solvents (petroleum benzene, chloroform, acetone, and alcohol) were greatly reduced (Table-2) due to their cent percent mortality of introduced insects on the 1st day of observation and the number of adult emerged were recorded minimum (1-2) or nil. Although, the moderate variation of mortality was observed between the treatment and control, the adult emergence in the treatment was reduced to a maximum number in acetone (10-33) followed by alcohol (75-148) and chloroform extracts (109-191). However, the lower concentration of 1000, 5000 and 10,000 ppm of petroleum benzene *Annona* extract recorded the highest adult emergence 360.33, 319.30 and 213.00, respectively while the control and acetone treated control registered 513.33 and 492.66 respectively.

The overall result on adult emergence of *Annona* seed extract revealed the efficiency of *Annona* seed extract (Petroleum benzene, Chloroform, Acetone & Alcohol) as an effective botanical protectant in arresting the pulse beetle population at higher concentration levels (1%,3%,5%).

Among the solvent used for seed extraction of *Annona*, the Acetone extract minimized the beetle population even at a minimum concentration (10-33).

Table-1. Percentage mortality of *C. analis* in Green Gram Treated with Annona Seed extracts

Extract	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Mean
Control	0.00 c (0.00d)	0.00 c (0.00d)	10.00a (18.43bc)	30.00a (33.21a)	10.00b (18.43bcd)	20.00ab (26.57a)	30.00a (33.21a)	0.00c (0.00b)	12.50 (16.23)
Acetone Control	0.00 c (0.00d)	10.00b (18.43abc)	0.00c (0.00d)	20.00ab (26.57ab)	30.00a (33.21a)	10.00b (18.43abc)	30.00a (33.21a)	0.00c (0.00b)	12.50 (16.23)
Petroleum benzene 1000 ppm	13.33ab (21.14ab)	6.67bc (12.28bcd)	20.00a (26.07abc)	20.00a (26.56ab)	20.00a (26.67abc)	16.67ab (23.85ab)	3.33c (6.14c)	0.00c (0.00b)	12.50 (17.76)
5000 ppm	10.00b (15.00bc)	23.33a (28.79a)	20.00ab (26.07abc)	20.00ab (26.07ab)	20.00ab (26.07abc)	6.67cd (12.29bcd)	0.00d (0.00c)	0.00d (0.00b)	12.50 (16.79)
10000 ppm	30.00a (32.21a)	6.67bc (8.85cd)	26.67a (30.99ab)	26.67a (0.79a)	10.00b (15.00cd)	0.00c (.00d)	0.00c (0.00c)	0.00c (0.00b)	12.50 (14.73)
Chloroform 1000 ppm	3.33cd (6.14cd)	26.67a (30.79a)	10.00bc (15.00c)	23.33a (28.79ab)	20.00ab (25.37abc)	13.00ab (21.14ab)	3.33cd (6.14c)	0.00d (0.00b)	12.50 (16.67)
5000 ppm	13.33bc (17.21bc)	26.67a (30.99)	23.33ab (28.79abc)	20.00ab (26.07ab)	6.67cd (12.29cd)	6.67cd (12.29bcd)	3.33cd (6.14c)	0.00d (0.00b)	12.50 (16.72)
10000 ppm	23.33ab (20.07ab)	20.00ab (26.07ab)	36.67a (37.22a)	16.67b (23.37ab)	3.33c (6.14d)	0.00c (0.00d)	0.00c (0.00c)	0.00c (0.00b)	12.50 (15.10)
Acetone 1000 ppm	0.00b (0.00d)	13.33a (21.14abc)	10.00a (15.00c)	10.00a (15.00b)	16.67a (23.36abc)	16.67a (23.85ab)	13.33a (21.14ab)	20.00a (26.67a)	12.50 (18.19)
5000 ppm	0.00c (0.00d)	13.33ab (21.14abc)	16.67ab (19.92bc)	20.00ab (26.07ab)	10.00ab (18.43bcd)	6.67bc (12.29bcd)	23.33a (28.29a)	10.0ab (15.00a)	12.50 (17.21)
10000 ppm	0.00c (0.00d)	16.67ab (23.85ab)	16.67ab (23.85abc)	23.33a (28.29ab)	26.67a (30.99ab)	10.00ab (18.43abc)	6.67bc (12.29bc)	0.00c (0.00b)	12.50 (17.21)
Alcohol 1000 ppm	13.33a (17.21bc)	16.67a (23.86ab)	13.33a (17.21bc)	16.67a (23.85ab)	20.00a (26.07abc)	13.33a (21.14ab)	13.33a (21.14ab)	0.00b (0.00b)	13.33 (18.81)
5000 ppm	23.33a (28.79ab)	16.67ab (23.37ab)	26.67a (30.99ab)	23.33a (28.79ab)	6.67bc (12.19cd)	3.33c (6.14cd)	3.33c (6.14c)	0.00c (0.00b)	12.91 (17.06)
10000 ppm	20.00ab (26.07ab)	13.33b (17.71abc)	36.67a (36.84a)	20.00ab (26.07ab)	10.00b (15.00cd)	0.00c (0.00b)	0.00c (0.00c)	0.00c (0.00b)	12.50 (15.21)
MEAN	10.71 (13.70)	15.00 (20.52)	19.04 (23.31)	20.71 (26.39)	15.00 (20.62)	8.81 (14.03)	9.28 (12.41)	2.14 (2.93)	12.58 (16.74)

	SED	CD (0.05)	CD (0.01)
Days	1.68681	3.32749	4.38339
Treatment	2.23144	4.40186	5.79868
Days*treatment	6.31146	12.45033	16.40116

ppm – parts per million
SED=Standard Deviation
CD=Criticaldifference
Means with same letter are onpar with each other

Table-2. Adult emergence in green gram treated with Annona seed extracts

	EXTRACT						
Treatment (T)	1000ppm	5000ppm	10,000ppm	1 %	3%	5%	T-mean
Control	513.33 a	513.33 a	513.33 a	513.33 a	513.33 a	513.33 a	513.33 a
Acetone control	492.66 a	492.66 a	492.66 a	492.66 a	492.66 a	492.66 a	492.66 a
Petroleum benzene	360.33 b	319.33 b	213.00 b	2.00 b	0.00 b	0.00 b	149.11
Chloroform	191.66 c	139.33 c	109.00 c	1.00 b	0.00 b	0.00 b	73.50
Acetone	33.00 d	24.00 d	10.00 d	0.00 b	0.00 b	0.00 b	11.16
Alcohol	148.33 c	123.66 c	75.33 c	0.66 b	0.00 b	0.00 b	58.00
E-Mean	289.88	268.72	235.55	168.27	167.66	167.66	216.29

	SED	CD (0.05)	CD (0.01)
Days	9.54	19.03	25.27
Treatment	9.54	19.03	25.27
Days * treatment	2.06	46.63	61.91

ppm – parts per million SED=Standard Deviation
CD=Critical difference Means with same letter are on par with each other

The moderated insecticidal effect was noticed for alcohol and chloroform extract as evidenced from 75-191 of adult emergence.

Impact of Annona seed extract on percentage weight loss of Green Gram by C.analis :

A similar trend of observation was noticed for percentage weight loss as seen in adult emergence. Dose-dependent variation among the treatment were also noticed in this parameter as like that of adult emergence.

Minimum percentage of weight loss of green gram were recorded for higher dose levels of all the solvent of *Annona* extracts (0.04 - 0.01%). The weight loss of the grain was also reduced to a minimum level in acetone extract at a lower concentration (0.60%-2.08%). This was followed by alcohol (4.73% -8.01%) and chloroform (8.84%-21.78%) of moderateloss in weight. The 1000 and 5000 ppm of Petroleum benzene *Annona* extract recorded the highest adult emergence among the treatment (51.84- 95.00%), which were on par each that of control and acetone treated control.

From this table-3, it was evident that the higher concentration of *Annona* seed extract possesses the potential insecticidal activity, reducing the weight loss at minimum level (0.04-0.11%). among the different solvents used, the acetone ranks first, evidenced by minimum adult emergence and weight loss even at lower concentrations (1000, 5000 and 10000 ppm) followed by alcohol and chloroform.

Impact of Annona seed extract on germination percentage and Vigor index in green gram :

Germination percentage between control, acetone treated control and treatments

Table-3. Percentage Weight loss of green gram treated with Annona seed extracts

	CONCENTRATIONS						
Treatment	1000 ppm	5000 ppm	10,000 ppm	1%	3%	5%	T-Mean
Control	59.43 a (50.43) a	59.43 a (50.43) a	59.43 a (50.43) a	59.43 a (50.43) a	59.43 a (50.43) a	59.43 a (50.43) a	59.43 (50.43)
Acetone Control	58.87 a (50.11) a	58.87 a (50.11) a	58.87 a (50.11) a	58.87 a (50.11) a	58.87 a (50.11) a	58.87 a (50.11) a	58.87 (50.11)
Petroleum benzene	51.84 a (46.05) b	55.00 a (47.88) a	37.05 b (37.44) b	0.11 c (1.86) b	0.05 c (1.30) b	0.04 c (1.15) b	24.01 (22.62)
Chloroform	21.78 a (27.42) c	15.16 b (22.28) b	8.84 c (16.48) c	0.10 d (1.81) b	0.05 d (1.30) b	0.04 d (1.15) b	7.66 11.74
Acetone	2.08 a (8.12) d	1.57 a (7.17) c	0.60 ab (4.27) d	0.04 b (1.15) b	0.06 b (1.46) b	0.04 b (1.15) b	0.733 (3.89)
Alcohol	18.01 a (24.93) c	4.73 c (20.01) b	4.73 c (12.52) c	0.07 d (1.46) b	0.07 d (1.46) b	0.04 d (1.15) b	5.81 (10.26)
E-Mean	35.34 (34.51)	33.66 (32.98)	28.25 (28.55)	19.77 (17.80)	19.76 (17.68)	19.74 (17.52)	26.08 (24.84)

	SED	CD (0.05)	CD (0.01)
Days	0.84	1.68	2.23
Treatment	0.84	1.68	2.23
Days * treatment	2.06	4.12	5.47

ppm – parts per million
SED=Standard Deviation
CD=Criticaldifference
Means with same letter are on par with each other

Table-4. Germination percentage and vigour index

Treatments	Ranks	Means
Control	5	573.95 e
Acetone control	6	463.30 f
Petroleum benzene	4	702.94 d
Chloroform	3	1732.60 c
Acetone	1	2024.20 a
Alcohol	2	1880.00 b

Table 5 Phytochemical screening of Annona seed extracts

Compounds	Petroleum Benzene	Chloroform	Acetone	Alcohol
ALKALOIDS				
Wagners test	+	-	-	+
Mayer's test	-	+	+	-
Dragondroffs test	+	+	+	-
FLAVANOIDS	-	-	-	+
SAPONINS	+	+	+	+
ANTHOCYANIN	-	+	+	+
TANNINS	-	-	+	+
TERPENOIDS	-	-	+	+
PHENOL	+	+	+	+
GLYCOSIDASE	+	-	-	+
CARBOHYDRATE				
Molisch's test	+	+	+	+
Benedict's test	+	+	-	+
PROTEINS	+	+	+	+

"+" indicates Present, "-" indicates absent

varied significantly (Table-4). Therefore the 100% was recorded for all the treatments at lower and higher concentrations (7.33 – 83.33), except petroleum benzene at lower concentrations. As the insect infestation in control and acetone treated control recorded maximum, the germination percentage also got reduced.

As the germination percentage was high for all the treatments both at lower and higher concentration, the vigor of seedlings also high. The vigor index was reduced to a greater extent control and acetone treated control.

Phytochemical screening of Annona seed extract :

The result on phytochemical screening of *Annona* seed extract were tabulated in table 5.Phytochemical screening was carried out in petroleum benzene, chloroform, acetone and alcohol extracts for the presence of Alkaloids, flavonoids, terpenoids, saponins, tannin, anthocyanin, glycoside, phenol, carbohydrate and proteins. The compounds carbohydrate, protein, saponins and phenol were present in all the extract. The numbers of compounds were maximum in alcohol extract, (alkaloids, flavonoids, anthocyanin, terpenoids, saponins, Tannins, phenol, carbohydrate) followed by acetone extract (Alkaloids, terpenoids, saponins, tannin, anthocyanin, glycoside, phenol, carbohydrate and protein). In comparison with alcohol and acetone extract the chloroform and petroleum benzene extracts were found to possess the minimum compounds (Alkaloids, saponins, phenol, carbohydrate and protein) and (Alkaloids, terpenoids, saponins, tannin, anthocyanin, phenol, carbohydrate and protein) respectively.

Many researchers carried out phytochemical screening of *Annona*. Annonaceae are empirically known to elicit insecticidal activities[1]. Plant species in this family contain an array of toxic compounds such as acetogenins, alkaloids, flavonoids that confer to these plants their insecticidal proprieties [4]. Both seeds of *A. squamosa*and *A. muricata* contain a great amount of acetogenins.

The higher concentration of all the extracts of *Annona* and lower concentration of acetone produced maximum mortality, minimum adult or no emergence and weight loss, higher germination percentage, and vigor index forced as to recommend. This acts as a bioprotectant to the rural farmers to keep the grain healthy. The test plant *Annona squamosa* has a wide geographical distribution, integrating and promoting its use as an alternative plant-based method in stored pest control. If appropriately used at an effective dose, the extracts of *Annona* have been revealed to be a low cost, powerful eco-friendly insecticide, thereby avoiding the accumulation of non-target endofauna.

References :

1. Das N.G., D. Goswami and B. Rabha (2007) *J Vector Borne Dis*; *44:* 145–148.
2. Dharmasena C. M. D., W. M. Blaney and M.S.J. Simmonds (2001). *Phytoparasitica 29:* 191 - 196.
3. George, D., and R. Pamplona (1999). *Editional Safelize Spain*, *1:* 381.
4. Grzybowski A., M. Tiboni, M.A. da Silva, R.F. Chitolina, M. Passons and J.D. Fontana (2011) *Rev Bras Farmacogn*; doi: 10.1590/S0102-695X2012005000026
5. Harris, K. L., and C. J. Lindblad (1978). *America Association of Cereal Chemist*, *193*:
6. Kaushik R. and P. Saini (2009) *J Exp Zool*, *12:* 395-398.
7. Lalitharani, S., V. Kalpanadevi and V. R. Mohan (2013). *Bioscience discovery*, *4*(1): 5-11.
8. Naik, G. M., P. Abhirami and N. Venkatachalapathy (2020) *Springer, Cham*. DOI: 10.1007/978-3-030-41376-7_12.
9. Oghbaei, M. and J. Prakash (2017) *Journal of food science and technology*, *54*(4): 871-879 DOI:10.1007/s13197-016-2382-x
10. Pier, O. (2008). *United States Geological Survey and United States Forest Service: Pier species lists*, *8*.
11. Sheela, D. and F. Uthayakumari (2013). *Bioscience discovery*, *4*(1): 47-53.
12. Sofowora A. (1993) *Medicinal Plants and Traditional Medicinal in Africa.* 2nd Ed. Sunshine House, Ibadan, Nigeria: *Spectrum Books Ltd; Screening Plants for Bioactive Agents;* pp. 134–156.
13. Sonakamble M.M., B.K. Dhanorkar, A.T. Munde and A. M. Saonkamble (2000) *J. Soils crop 10:* 236-239.
14. Trease G. E. and W. C. Evans (2002) *Pharmacognosy*. 15th Ed. *London: Saunders Publishers;* . pp. 42–44. 221–229, 246–249, 304–306, 331–332, 391–393.
15. Tresina, P. S., S. M. J. Kala and V. R. Mohan (2012). *Journal of Applied Pharmaceutical Science*, *2*(12): 112-124.