



Formulation of the leaves extract of *Syzygium cumini* (Jambhul) targets to *Callosobruchus maculatus* so as to reserve the food grains

Atmaram V Andhale

Department of Zoology, Nowrosjee Wadia College, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India

Abstract

The pest is usually applied to signify a species of insects destructive to cultivated plants and stored grains. A pest is an organism whose population often increase above a certain level of economic injury and its existence conflict with man's welfare convenience and profits. When economic injury level is greater need to control the pest damage enforcing biopesticides rather than disadvantages of chemical pesticides to conserve the domestic grains. Looking toward all referred medicinal benefits of *Syzygium cumini* (Jambhul) was selected as precursor for the biopesticide formulations for the preservation of domestic food grains, the *Vigna aconitifolius* (Moth bean) and *Vigna radiates* (Mung bean). Results from study reveals that extract of leaves *Syzygium cumini* (jambhul) in chloroform and acetone is effective counter to *Callosobruchus maculatus* which help in preservations *Vigna aconitifolia* (Mataki) and *Vigna radiata* (Mung) so that we save its nutritive values. The tabulated data studied by using Method of Lowry's *et al.*, (1951 for protein shown that losses from respective grains were decline as concentrations of screened bio-pesticide increased. Along with pesticidal properties the refereed medicinal importance of plant, *Syzygium cumini* contract additional benefits to human food.

Keywords: *Syzygium cumini* (Jambhul), *Callosobruchus maculatus*, *Vigna aconitifolius* (Moth bean) and *Vigna radiates* (Mung bean) and Soxhlet apparatus

Introduction

Looking toward all referred medicinal benefits *Syzygium cumini* (Jambhul) we have selected as precursor for the biopesticide formulations for the preservation of domestic food grains, the *Vigna aconitifolius* (Moth bean) and *Vigna radiates* (Mung bean). As we know that worldwide there is great loss of many food grains. According to the National Institute of Environmental Health Sciences, pesticides have as yet incompletely understood effects on humans. The National Alliance for Pesticide-Free Lawns says, out of 30 commonly used lawn pesticides, 19 are linked with cancer or carcinogenicity, 13 are linked with birth defects, 21 with reproductive effects, 26 with liver or kidney damage, 15 with neurotoxicity, and 11 with disruption of the endocrine system (Wikipedia). And if the chemical pesticides were used as preservative material directly food stuff, then possibility of adverse effect on human life. IPM practices are mostly directed toward detecting and controlling pests inside storage facilities or within the commodity, not in the outdoor vicinity of the store (Dowdy and Mcgaughey, 1994) [4]. Yet some of the stored-product pests are highly mobile and temporarily and spatially patchy in distribution; therefore, information on pest population occurrence, ecology and behavior in the vicinity of storage facilities is important, both for effective monitoring and controlling of stored-product pests (Campbell *et al.*, 2002) [1].

Bean beetles (cowpea seed beetles), *Callosobruchus maculatus*, are agricultural pest insects of Africa and Asia. Females lay their eggs on the surface of beans (Family Fabaceae). Eggs are deposited (=oviposition) singly and several days after oviposition, a beetle larva (maggot) burrows into the bean. At 30°C, pupation and emergence of an adult beetle occurs 21-30 days after an egg was deposited. Adults are mature 24 - 36 hours after emergence and they do not need to feed. Adults may live for 1-2 weeks during which time mating and ovipositor occurs.

Since larvae cannot move from the bean on which an egg was deposited, the oviposition choice of a female determines the future food resources available to their offspring. As a result, it is the most critical choice a female makes for her offspring, because it will influence their growth, survival and future reproduction (Mitchell, 1975; Wasserman and Futuyma, 1981) [13, 15]. Although females can be induced to lay eggs (oviposit) on a wide range of bean species, very few bean species result in normal development and the successful emergence of adults. Some bean species are very clearly toxic to *Callosobruchus maculatus* larvae (Janzen 1977) [9].

Bean beetles exhibit two adult forms (morphs), a sedentary (flightless) form and a dispersal (flying) form. The dispersal morph is induced by high larval density in stored beans or laboratory cultures, and is caused by density dependent micro-habitat temperature increases (Utida 1956, 1972,) [14].

In Nigeria, *Callosobruchus maculatus* (Fabricius) is the major primary pest of stored seeds of cowpea and it causes a wide spread damage which constitutes a major constraint on the availability of this legume (Ofuya and Credland, 1995). The control of this brunched is largely dependent on different conventional insecticides that

were being used indiscriminately by local farmers and marketers, an act that can lead to the evolution of resistance in *C. maculatus*.

Looking towards drawbacks of traditional pesticides, now day's practices of bio-pesticide come forward along with the modern or sustainable development in bio world.

Materials and Methods

Non infected grains of the *Vigna aconitifolius* (Moth bean) and *Vigna radiates* (Mung bean) were purchased from the local market and were screened for the damaged and infected grains if any. The noninfected grains were washed in clean water and were dried at 45 °C in the oven to kill if any infective stage of the pest exists in the grains. The dry grains were placed in the clean glass bottle. The cap of the bottle was perforated for the ventilation. Wet muslin cloth was tied at the mouth of the bottle before capping to maintain the humidity for the survival of the pest.

Infected pulses were obtained from the market and were identified at the agriculture college of Aurangabad. Two species of the pulse beetles were obtained. Some were *Callosobruchus chinensis* and some were *Callosobruchus maculatus*. *Callosobruchus chinensis* were separated from the infected grains. Ten males and 10 females were released in the bottle containing 500 grams of the grains of *Phaseolus radiatus* and 500 grams of grains of *Phaseolus aconitifolius* were allowed to grow. The adult females laid the eggs on the grains. Beetles when died were removed and the culture was maintained at room temperature. After about 28 days, the young adults have begun to emerge out from the grains. The adults hatched were used for the experimentation and some were released in the fresh pulses again to maintain the stock culture.

The fresh leaves of *Syzygium cumini* (jambul) collected from Nowrojee wadia college campus and were dried in the shade then in the oven at 45 °C. The dry leaves were ground to make the powder. The powders of all plant materials were stored in the airtight polyethylene bags. Ten grams of each of the dried powders were packed in filter paper and extract was extracted in Soxhlet apparatus in 1:10 ratio i.e. 10 gm powder in 100 ml solvent. After eight hours of continuous extraction the final extract was kept open to evaporate the solvent and remaining as stock solution extract was stored at 4°C in a refrigerator until use. Extracts of plant material were extracted in chloroform, acetone, and water were stored after evaporation of solvent in refrigerator. 24 grams of the disinfected pulse grains were taken in each of the glass bottle. One bottle was maintained as control and others were labeled and were used for experiment. Same procedure for the grains of *Phaseolus radiates*. 0.1 ml extract of leaves of *Syzygium cumini*, was dissolved in respective solvent to make 10 ml volume and from this diluted extract 0.4 ml, 0.8 ml, 1.2 ml, 1.6 ml diluted extract was added in each bottle containing 24 gms grains. The bottles were tied up with muslin cloth and were kept open for 48 hours in well ventilated room to evaporate the chloroform. Same procedure was adapted for extracts of other plant materials in different solvents. After 48 hours freshly hatched five males and five females from stock were released in each of the control and experimental bottles. The *Callosobruchus chinensis* beetles moved towards the mouth of the bottle and tried to live near the mouth of the bottle. Regarding the aqueous extracts, 10-gram powder was soaked in 50 ml distilled water for 24 hours. The extract was filtered and the filtrate was used for experiment.

The mortality rate of adults released was observed for 24 hours and 96 hours of the exposure in different concentrations of different plant extracts and was recorded. The live beetles laid and glued the eggs on the grains. The dead adults were removed. The adults survived for about 10 days during which period they laid the eggs. After the death of all adults, the number of eggs layed in each of the control and experimental bottle were counted the culture was allowed to grow. It took nearly 28 days to hatch the adults. The adults hatched were counted and were removed from the culture. After about 40 days of the release of the adults in the culture, emergence of the adult stopped. Then the infected grains in each bottle were counted and recorded. Weight of 100 infected grains from each bottle was taken and recorded. Where the number of infected eggs was less, the available infected eggs from the respective set was weighed and converted to that of 100 eggs. Similarly, weight of 100 noninfected grains was taken and recorded. 24 gms of the seeds of *Phaseolus aconitifolius* contained on an average 1000 grains and 24 gms of the seeds of *Phaseolus radiates* contained on an average 500 grains.

Protein contents of noninfected grains and hollow grains from different experimental sets were estimated by the method of Lowry's *et al.*, (1951). The data was compiled in the tables and the calculations were done by following formulae.

$$\text{Percent weight loss in infected grains} = \frac{100 \times (\text{Wt. of 100 solid grains} - \text{Wt. of 100 hollow grains})}{\text{Wt. of 100 solid grains}}$$

$$\text{Percent weight loss in total grains} = \frac{\text{Number of infected grains} \times (\text{Wt. of 100 solid grains} - \text{Wt. of 100 hollow grains})}{\text{Total weight of grains}}$$

$$\text{Percent protein loss in infected grains} = \frac{100 \times (\% \text{ Protein contents of solid grains} - \% \text{ Protein contents of hollow grains})}{\% \text{ Protein contents of solid grains}}$$

$$\text{Percent protein loss in total grains} = \frac{\left[\frac{\text{Number of infected grains} \times \text{Wt. of hundred solid grains} \times \text{Protein loss in infected grains}}{100} \right]}{\text{Initial weight of total grains}}$$

Observation and Results

Vigna aconitifolia and *Vigna radiata* are the common pulses used as sprouted grains for several preparations of breakfast and meals in the country. It is highly susceptible to the infestation of the bruchids, *Callosobruchus chinensis* and *Callosobruchus maculatus* (Plate-I). General observations show that *vigna aconitifolia* and *vigna radiata* seeds if remains for few months in the house are heavily attacked by these pests and makes them unfit for eating. *Callosobruchus maculatus* occurs commonly in most of the pulses (as in Plate II).

The female lay eggs and glues on the grains (Plate III). The number of eggs glued on single grain varies from one to five (Plate III). The female laid eggs for about 8 to 10 days. The tiny larvae that hatches out penetrates in the grains and grows within the grains. They eat the grains from inside and were makes them hollow and then pupates. The pupa can be removed from the grain. After about 28 days, the adult emerges out from the grains.

Leaves of *Syzygium cumini* plant (Plate I) were collected and photographed. The extracts of the powder in different solvents after evaporation of the solvent were in the form of thick oily or wet materials. Plate III shows the experimental set up and the plate shows variable number of eggs of *Callosobruchus maculatus* glued to the grains.

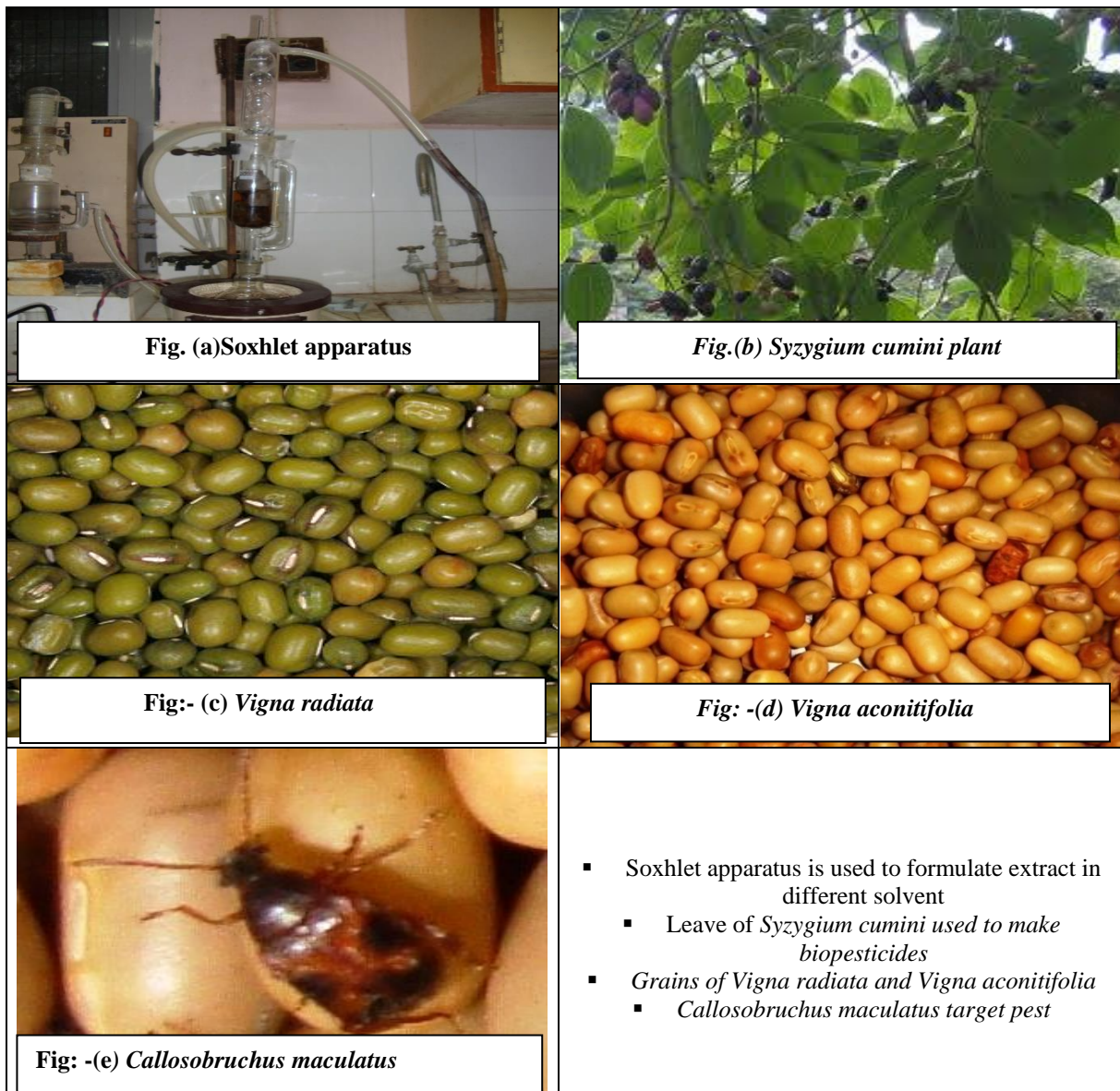


Plate 1: (shown that Materials selected for Experiment)



Experimental set up *Vigna aconitifolia*



Experimental set up *Vigna radiata*



Number of eggs laid on *Vigna aconitifolia* grains by *Callosobruchus maculatus*



Vigna radiata* grains with infestation of *Callosobruchus maculatus

Plate II: (shown the obsevation to experimental set up)



***Vigna aconitifolia* grains before experiment**



Vigna aconitifolia* grains from control after 40 days of release of five pairs of *Callosobruchus maculatus



Plate III: (shows infestation of grains by *Callosobruchus maculatus*)

Table 1.1 : Effect of chloroform extract *Syzygium cumini* (Jambhul) leaves on the reproductive activity of the stored grains, *Vigna radata* (mung) pest, *Callosobruchus maculatus*

Dose in ml/kg	Mortality		Number of eggs laid	Infected grains	Emergence of Adult
	24hr	96hr	Till death of adults	Till death of adults	Up to 40 days
Control	Nil	Nil	396	158	106
0.4	1	1	63	27	14
0.8	1	2	37	19	7
1.2	3	2	23	11	3
1.6	5	3	13	3	Nil

Table 1.2: Effect of acetone extract of *Syzygium cumini* (Jambhul) leaves on the reproductive activity of the stored grains-*Vigna radata* (mung) pest, *Callosobruchus maculatus*.

Dose in ml/kg	Mortality		Number of eggs laid	Infected grains	Emergence of Adult
	24hr	96hr	Till death of adults	Till death of adults	Up to 40 days
Control	Nil	Nil	200	175	92
0.4	1	1	67	13	8
0.8	2	4	59	11	6
1.2	4	3	27	Nil	Nil
1.6	4	6	10	Nil	Nil

Table 1.3: Effect of aqueous extract of *Syzygium cumini* (Jambhul) leaves on the reproductive activity of the stored grains-*Vigna radata* (mung) pest, *Callosobruchus maculatus*.

Dose in ml/kg	Mortality		Number of eggs laid	Infected grains	Emergence of Adult
	24hr	96hr	Till death of adults	Till death of adults	Up to 40 days
Control	Nil	Nil	434	212	122
0.4	Nil	Nil	139	127	97
0.8	Nil	1	126	106	86
1.2	Nil	2	98	75	67
1.6	2	2	87	67	49

Table 1.4: Effect of chloroform extract of *Syzygium cumini* (Jambhul) leaves on the reproductive activity of the stored, *Vigna aconitifolia* (moth bean) pest, *Callosobruchus maculatus*.

Dose in ml/kg	Mortality		Number of eggs laid	Infected grains	Emergence of Adult
	24hr	96hr	Till death of adults	Till death of adults	Up to 40 days
Control	Nil	Nil	476	155	136
0.4	1	3	126	63	25
0.8	1	4	76	28	12

1.2	2	5	39	18	05
1.6	3	5	23	07	00

Table 1.5: Effect of acetone extract of *Syzygium cumini* (Jambhul) leaves on the reproductive activity of the stored grains, *Vigna aconitifolia* (moth bean) pest, *Callosobruchus maculatus*.

Dose in ml/kg	Mortality		Number of eggs laid	Infected grains	Emergence of Adult
	24hr	96hr	Till death of adults	Till death of adults	Up to 40 days
Control	Nil	Nil	430	132	122
0.4	2	3	134	58	14
0.8	3	4	96	41	09
1.2	3	4	52	20	00
1.6	5	4	22	00	00

Table 1.6: Effect of aqueous extract of *Syzygium cumini* (Jambhul) leaves on the reproductive activity of the stored, *Vigna aconitifolia* (moth bean) pest, *Callosobruchus maculatus*

Dose in ml/kg	Mortality		Number of eggs laid	Infected grains	Emergence of Adult
	24hr	96hr	Till death of adults	Till death of adults	Up to 40 days
Control	Nil	Nil	648	216	152
0.4	Nil	Nil	175	152	142
0.8	Nil	Nil	163	150	130
1.2	1	1	145	132	110
1.6	1	1	97	80	52

Table 2.1: Effect of acetone extract of *Syzygium cumini* (Jambhul) on the weight and protein loss in grains of *Phaseolus radiatus* by the stored grain pest, *Callosobruchus maculatus*

Dose (ml extract/Kg Grains)	Initial weight of grains (gm)	Total number of grains	Number of Infected grains in 40 days	Wt. of 100 solid grains (gm)	Wt. of 100 hollow grains	% Wt. loss in infected grains	% Wt. Loss in total grains	% Protein contents of solid grains	% Protein contents of hollow grains	% Protein loss in infected grains	% Protein loss in total grains
(A)	(B)		(C)	(D)	(E)	(F)	(G)	(H)	(I)	(J)	(K)
Control	24	512 ±32	175	4.995±0.11	0.565±0.12	88.68	32.30	27.54±1.30	8.32±0.50	69.78	32.29
0.4	24	512±32	13	4.995±0.11	2.58±0.08	48.34	1.308	27.54±1.30	12.39±1.00	55.01	1.308
0.8	24	512±32	11	4.995±0.11	2.921±0.09	41.54	0.950	27.54±1.30	20.26±0.11	26.43	0.950
1.2	24	512±32	Nil	4.995±0.11	-			27.54±1.30	-	-	0.00
1.6	24	512±32	Nil	4.995±0.11	-			27.54±1.30	-	-	0.00

± indicates S. D. of three observations

Table 2.2: Effect of acetone extract of *Syzygium cumini* (Jambhul) on the weight and protein loss in grains of *Vigna aconitifolia* by the stored grain pest, *Callosobruchus maculatus*

Dose (ml extract/Kg Grains)	Initial weight of grains (gm)	Total number of grains	Number of Infected grains in 40 days	Wt. of 100 solid grains (gm)	Wt. of 100 hollow grains	% Wt. loss in infected grains	% Wt. Loss in total grains	% Protein contents of solid grains	% Protein contents of hollow grains	% Protein loss in infected grains	% Protein loss in total grains
(A)	(B)		(C)	(D)	(E)	(F)	(G)	(H)	(I)	(J)	(K)
Control	24	1085 ±42	132	2.312±0.12	0.325±0.15	85.94	10.92	23.82±1.30	8.65±0.53	63.68	10.92
0.4	24	1085±42	58	2.312±0.12	1.26±0.06	45.50	2.54	23.82±1.30	14.23±1.20	0.26	2.54
0.8	24	1085±42	41	2.312±0.12	1.20±0.03	48.09	1.89	23.82±1.30	15.27±0.30	35.89	1.89
1.2	24	1085±42	20	2.312±0.12	1.12±0.12	51.55	0.99	23.82±1.30	19.16±0.06	19.56	0.99
1.6	2	1085±42	Nil	2.312±0.12	-			23.82±1.30			-

± indicates S. D. of three observations.

The grains of *Vigna aconitifolia* and *Vigna radiata* before releasing the *Callosobruchus maculatus* males and females and after 40 days in control set are given in the plate II. The photograph shows the damaged hollow grains in large number.

The Effect of extracts of leaves of *Syzygium cumini* different solvents were showed variable damages and data were collected and tabulated in the form of different tables (1.1 to 1.6 and 2.1 to 2.2) It has been observed that all eggs laid do not hatch in to larvae and infect the grains and also all larvae which have infested the grains do not grow to emerge out as adults as it is observed in the tables.

In control of *Vigna radiata*, out of 396 eggs laid only 158 has infested the grains, and out of 147 only 106 adults emerged out (Table 1.1) and in control of *Vigna aconitifolia*, out of 476 eggs laid only 155 has infested the grains, and out of 143 only 136 adults emerged out (Table 1.5). It shows that there is low viability of the stages in life cycle of *Callosobruchus maculatus*.

Tables 1.1 to 1.3 shows the effect of chloroform, acetone, and aqueous extracts of leaves of *Syzygium cumini* on the mortality of adult *Callosobruchus maculatus* and their life cycle stages in the *Vigna radiata*. On other hand Tables 1.4 to 1.6 shows the effect of chloroform, acetone, and aqueous extracts of leaves of *Syzygium cumini* on the mortality of adult *Callosobruchus maculatus* and their life cycle stages in the *Vigna aconitifolia*.

Tables 1.1 to 1.6 shows the effect of chloroform, acetone, and aqueous extracts of leaves of *Syzygium cumini* on the mortality of adult *Callosobruchus maculatus* and their life cycle stages in the *Vigna radiata* and *Vigna aconitifolia* respectively. The mortality rate was high in chloroform and acetone extracts, and the number of eggs laid, grains infested and adults emerged were highly reduced with the increase in the concentration of the extract as shown in the tables and figures 1.1 to 1.6 There was no infestation and emergence of adults in the concentrations of chloroform extracts above 1.2 ml per Kg grains.

Aqueous extracts as shown in tables 1.3 and 1.6 had very poor toxic effects and poor inhibitory effect on the reproductive activity and hence fairly large number of the adults which emerged out can damage the pulses severely in the forthcoming generations.

The levels of the damages caused by the *Callosobruchus maculatus* in the control and the treated grains of *Vigna aconitifolia* and *Vigna radiata* were calculated and are given in the tables from 2.1 to 2.2. Table 1.2 shown the effect of acetone extract of *Syzygium cumini* (Jambhul) on the weight and protein loss in grains of *Phaseolus radiatus* by the stored grain pest, *Callosobruchus maculatus*. In control setup % of protein loss was 32.92 but in treated setup as we increase concentrations of acetone extract of *Syzygium cumini* exposure to grains from 0.4 to 1.6 ml/ kg grains then there were decline in loss of % of protein in total grains. Also, in table no. 2.2 Effect of acetone extract of *Syzygium cumini* (Jambhul) on the weight and protein loss in grains of *Vigna aconitifolia* by the stored grain pest, *Callosobruchus maculatus*. 10.92 Protein % loss in total no grains of *Vigna aconitifolia* and there were decline in this loss of protein content as we increase exposer concentrations of biopesticide made from *Syzygium cumini* shown that there was minimum loss in protein contents of grains.

Discussion

Natural compounds of plant origin are biodegradable, often of low mammalian toxicity, and pose low danger to the environment if used in small amounts. Recent research has focused on natural product alternatives for pest control in developing countries. Various studies have demonstrated the efficacy of neem (*Azadirachta indica* A. Juss.: Meliaceae) as a protectant (Makanjuola, 1989; Lale and Abdurahman, 1999; Golob and Gudrups, 1999).

Islam (1987)^[6] found that methanol and ethanol extracts of *A. squamosa* caused mortality of beetles starting one hour after their exposure period (42.5% for methanol and 45% for ethanol Mortality reached 100% within 24 h (methanol) and 4h (ethanol), respectively.

Plant extracts from native Omani flora, or introduced plants that are extensively cultivated were tested for their effectiveness in disrupting the life cycle of *C. chinensis*. The seed dressed with commercial neem caused fewer eggs to be laid compared with the ethanol control and the effect was less than that caused by a fresh neem preparation and an extract of *A. squamosa* in ethanol. Islam (1987)^[6] reported that the extracts of *Annona squamosa* were highly effective as antifeedant and as growth regulators.

Weight change in bruchids infested stored legumes was depicted if the storage period increased and significant changes in weight were observed (Modgil and Mehta, 1997). First there was an increase in the weight of infested grains up to one month. Subsequently it decreased. Decrease was the highest in mug bean, after 6 months of storage, when compared with the other two legumes. There was an increase in insect infestation with an increase in storage time, thereby, resulting in weight losses.

Conclusion

In present investigation study it was found that extract of leaves *Syzygium cumini* (jambhul) in chloroform and acetone is effective counter to *Callosobruchus maculatus* which help in preservations *Vigna aconitifolia* (Mataki) and *Vigna radiata* (Mung) so that we save its nutritive values. The data shows that protein losses from respective grains were decline as concentrations of screened bio-pesticide increased. Along that not only preservation of domestic grains but also add refereed medicinal importance of plant, *Syzygium cumini* in well-defined diet of humans.

References

1. Campbell JF, Mullen MA, Dowdy AK. "Monitoring stored-product pests in food processing plants: a case study using pheromone trapping, contour mapping and mark-recapture". *Journal of Economic Entomology*,2002;95:1089–1101.
2. Chopra RN, Nayar SL, Chopra IC. "Glossary of Indian Medicinal Plants". CISR, New Delhi, 1956, 225.
3. Copping LG. (ed.) "The Manual of Biocontrol Agents (*formerly the Biopesticide Manual*)". 3rd Ed. British Crop Production Council (BCPC), Farnham, Surrey, UK., 2004.
4. Dowdy AK, Mcgaughey WH. "Seasonal activity of stored-product insects in and around farm-stored wheat". *Journal of Economic*,1994;87:1351–1358.
5. Golob P, Webley O. J. "The use of plants and minerals as traditional protectants of stored products". *Bulletin of the Tropical Products institute of ODA, UK*, 1980, 12-13.
6. Islam BN. "Use of some extracts from Meliaceae and Annonaceae for control of rice hispa, *Dicladispa armigera*, and the pulse beetle, *Callosobruchus chinensis*. In: *Natural Pesticides from the Neem Tree (Azadirachta indica A. Juss.) and Other Tropical Plants.Proceedings of the 3rd International Neem Conference*, ed, 1987.
7. Jadhav KB, Jadhav LD. "Use of some vegetable oils, plant extracts and synthetic products as protectant from pulse beetle *Callosobruchus maculatus (F.)*". *Journal of Food Science and Technology*,1984;21:110-113.
8. Jain HK, Mehra KL. "Evolution, Adaptation, Relationships, and Uses of the Species of Vigna cultivated in India. In "Advances in Legume Science" eds. R.J. Summerfield and A.H. Bunting. Kew Royal Botanic Gardens", 1980, 459-468.
9. Janzen DH. "How southern cowpea weevil larvae (Bruchidae *Callosobruchus maculatus*) die on non-host seeds". *Ecology*,1977;58:921-927.
10. L.G. (ed.) "*The Manual of Biocontrol Agents (formerly the Biopesticide Manual)*". 4th Edition. British Crop Production Council (BCPC), Farnham, Surrey UK; 2009, 851.
11. Lale NES, HT Abdulrhman. " Evaluation of neem (*Azadirachta indica*) Ajuss seed oil obtained by different methods & neem powder for the management of *callosobruchus maculates (f)*, (coleptera bruchidae) stored prod. Res,1999;35:135-143.
12. Lale NES, HT Abdulrhman. "Evaluation of neem (*Azadirachta indica*) Ajuss seed oil obtained by different methods & neem powder for the management of *callosobruchus maculates (f)*, (coleptera bruchidae) stored prod. Res,1999;35:135-143.
13. Mitchell R. "The evolution of oviposition tactics in the bean weevil, *Callosobruchus maculates F*". *Ecology*,1975;56:696-702. National Institute of Environmental Health Sciences: Pesticides
14. Utida S. "Density dependent polymorphism in the adult of *Callosobruchus maculatus (Coleoptera, Bruchidae)*". *Journal of Stored Products Research*,1972;8:111-126.
15. Wasserman SS, DJ Futuyma. "Evolution of host plant utilization in laboratory populations of the southern cowpea weevil, *Callosobruchus maculatus* Fabrivius (Coleoptera: Bruchidae)". *Evolution*,1981;35:605-617.