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The nutritive values of food resolved by development of leaves extract from *Eucalyptus globulus* (Nilgiri) to Targets of *Callosobruchus maculatus*

Atmaram Vitthal Andhale
Assistant Professor in Department of Zoology, Modern Education Society's Nowrosjee Wadia College Pune, Savitribai Phule Pune University, Pune, Maharashtra, India

Abstract
To resolved infestations problems of many food grains, it is now necessary to develop eco-friendly and hygienic product to preserve the food products and avoids traditional negative impact of chemical pesticides. This approach was made for formulate of biopesticides from medicinally plant, *Eucalyptus globules* to control infestation of food grains *Vigna aconitifolius* and *Vigna radiates* from attack of beetles, *Callosobruchus maculatus*. The fresh leaves of *Eucalyptus globulus (nilgiri*) collected, dried and extract was prepared through Soxhlet apparatus in 1:10 ratio i.e., 10 gm powder in 100 ml of different solvent systems as chloroform, acetone, and water. The mortality rate of adults released was observed after 24 and 96 hours of the exposure against different concentrations of extracts from plant, Eucalyptus globulus. Protein contents of treated (hollow) and nontreated grains were estimated by the method of [1]. The data was tabulated and result recorded. The mortality of *Callosobruchus maculatus* from experimental and controlled set was recorded which shown that it is directly proportional to concentration of screened biopesticides from *Eucalyptus globules*. It was shown that protein losses from respective grains were deterioration against concentrations of partitioned bio-pesticide. Thus, in present investigation, it was successfully formulated bio-pesticide from plant leaves of Eucalyptus globulus (nilgiri) and avoids the economical and nutritive loss of food grains

Keywords: eucalyptus globulus (nilgiri); species: *C. maculatus*; food grains: *V. aconitifolius & V. radiat*es and apparatus-soxhlet

Introduction

Worldwide food grains are mostly infested by insects like beetles and excessive economical and nutritive losses to users. So, preservation of domestic food grains become challenging approach to farmers or operators. In order to resolved the problems of infections of food grains, present research approach was made to formulate the eco-friendly and hygienic biopesticides from medicinally important plant *Eucalaptus globules* to control influx of beetles, *C. maculatus in food* grains *V. aconitifolius* and *V. radiates.*

According to the [2] It is very challenging to understand the negative impact pesticides on human and their life and on interelement. Maximum people were exposed to a certain level of pesticides. E.g., Farmers who involvement routine contact to chemical pesticides have shown neurological symptoms such as headache and other symptoms. To avoid undesirable impact of traditional pesticides, now concept of IPM came forward. In IPM new approaches has to be develop and define biopesticide from natural resources.

Naturally defined biopesticides control the target pest through nonpoisonous mechanisms while the chemical or traditional pesticides kill the pest by toxic mechanisms and also kill non target species. Biopesticide is one of the key components of IPM. [3] Bio-pesticides are well-thought-out eco-friendly and easy to use. (EPA) In the USA, the EPA controls of earth friendly Insects. Various naturally-occurring materials like plant, Fungai, organisms have been defined as bio-pesticides [4].

Most of the traditional methods specially chemical pesticides were damage to the environment. Due to the very low cast and low very less environmental threats and low mammalian harmfulness, pest management is much more attentions of research through natural products [5].

Bean beetles, *C. maculatus* are most abundant agricultural pest insect which is having very high growth rate at optimum condition of storage facility of food grains [6, 7].

So, it is very challenging approach to develop naturally origin products to control the infection of food grains from insect attack. In present research setup approached to screen out or formulate bio pesticides from medicinally important plant, *Eucalyptus globulus (nilgiri*) (Nilgiri) for preservations of domestic food grains and targets to *Callosobruchus maculatus*

Materials and Methods

Fresh food grains of the *V. aconitifolius* and *V. radiates were* taken from market and were mark off the any damaged or infection by beetle. The uninfected grains were cleaned in water and were dried at 45^0 C. in the oven to kill if any infectious stage of the pest in or on the grains. The dry grains were located in the clean glass bottle and the cap of the bottle was punctured for the ventilation. Wet muslin cloth was knotted at the mouth of the bottle earlier capping to keep the humidity for the existence of the pest.

Pest-ridden pulses were obtained from the market and were identified at ZSI, Pune. Two species of the pulse beetles were got were *Callosobruchus chinensis* and some *Callosobruchus maculatus*.

Callosobruchus maculatus were detached from the infected grains. Ten males and 10 females were permitted in the bottle containing 500 grams of the grains of *Phaseolus radiatus* and 500 grams of grains of *Phaseolus aconitifolius* and allowed it to be grow. The adult female's pest placed the eggs on the grains. Beetles when deceased were detached and the culture was preserved at room temperature. Afterward about 28 days, the new adults have begun to develop out. The adults marked were used for the research and some were released in the fresh pulses again to continue the stock culture The medicinally important plant, commonly known as Nilgiris and scientifically known as *Eucalyptus globulus* or *E globulus* collected from Nowrojee Wadia College Campus and were dehydrated in the shade then dried in the oven at 45 ^{0}C. The dehydrated greeneries were crushed to make the residue. The residues of all plant materials were kept in the airtight polyethylene bags.

Defined 10 grams of each of the dehydrated residues were packed in filter paper and abstract was removed through Soxhlet Apparatus in 1:10 ratio (i.e., 10 gm powder in 100 ml different solvent). After continuous extraction process, the final removal was kept open to disappear the solvent. Remaining used as stock extract which was kept at 4ºC in a refrigerator further use. For removal of extract the different solvent system used were chloroform, acetone, and water.

The glass bottles containing disinfected grains of 24gms were taken one as control and another is an experimental. The bottles were knotted up with muslin cloth and were reserved open for 48 hours in well ventilated room to evaporate the solvents. The food grains of *V. aconitifolius* and *V.radiates* were used. 0.1 ml removal of greeneries of *Eucalyptus globulus***,** was dissolved in individual solvent to make 10 ml volume. From this, it was dilute to 0.4, 0.6 0.8, 1.2, 1.6 ml in respective solvents and was added in each bottle containing 24 gems grains. Afterwards 48 hours freshly hatched five males and five females from stock were pushed in each of the control and experimental bottles. The beetles, *C. maculatus* stimulated towards the mouth of the bottle and tried to live near the mouth of the bottle.

Suitable the aqueous abstracts, 10-gram powder was saturated in 50 ml distilled water for 24 hours. The removal was filtered and the filtrate was used for experiment setup.

In the experiment setup with control, the mortality rate of adults released was observed for 24 hours and 96 hours for the exposure in different concentrations of screened biopesticide from plant, *Eucalyptus globulus* and record was recorded. The animate beetles laid the eggs on the grains. The dead adults were detached. The adults lived for about 10 days during which they laid the eggs. After the death of all adults, the number of eggs laid in each of the control and experimental bottle were calculated the culture was allowed to grow further. It acquired nearly 28 days to hatch the adults. After hatching, the adults were counted and were removed from the culture. Subsequently about 40 days of the release of the adults in the culture, rise of the adult stopped.

Then the verminous grains in each bottle were calculated and noted. Weight of 100 verminous grains from each bottle was occupied and recorded. Likewise, weight of 100 clean grains was taken and verified. In 24 gms of the seeds of *Phaseolus aconitifolius* contained on an average 1000 grains while in 24 gms of the seeds of Phaseolus *radiates* contained on an average 500 grains.

Protein contents of clean grains and hollow grains (infested one) from different experimental sets were estimated by [1].

The experimental data was assembled in the tables and fig and the calculations were done by formulae as given below.

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

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

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

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

Results and Discussion

Results of formulated extract of *Eucalyptus globules* Leaves or greeneries on *Callosobruchus macultus* were tabularized in the tables from 1.1 to 1.6 and from 2.1 to 2.2 and in Photo plates as I, II and III in which fig. from 1 to 13 were given.

(a) *Plant Leaves of Eucalyptus globulus*

(b) *V. radiate*

(c) *V. aconitifolia*

(d) Instrument Soxhlet

(e) C. maculatus

Photo-Plate 1: fig. a to fig. e: Selected materials for experimental setups

(f) Experimental set up of *Vigna aconitifolia*

(g) Experimental set up *of Vigna radiata*

(h) shown eggs laid on *Vigna aconitifolia*

(i) infestation of *Vigna radiata* by *Callosobruchus maculatus*

Photo-Plate 2: fig. (f) to fig (i) were shown Experimental Setup

(j) *Vigna aconitifolia earlier of experiment*

(k). Shown contamination of Vigna aconitifolia grains from experimental setup after 40 days of issue of five pairs of Callosobruchus maculatus

Fig (l). *Vigna radiata grains earlier of experiment*

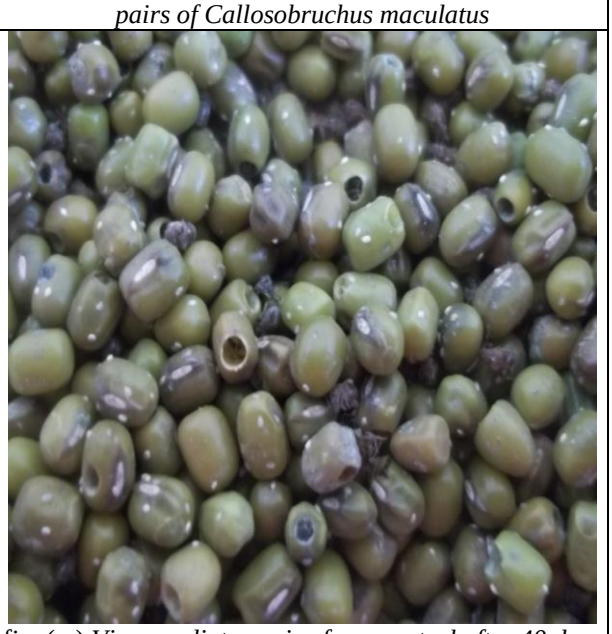

fig. (m) *Vigna radiata grains from control after 40 days of issue of five pairs of Callosobruchus maculatus*

Photo-Plate 3: Fig. (j) to fig (l): shown Controlled (non-exposed) and experimental (exposed) to *C. maculates invasion limits to food grains.*

Table 1: shown treatment of removal of *E. globules greeneries* in chloroform *of* on the multiplicative activity of the *C. maculates in* stored grains-*V. radata*.

Dosage in ml/kg	Death Against Exposure		Number of eggs placed	Infested grains	Emergence of Adults
	After 24hr	After 96hr	Till death of adults	Till death of adults	Up to 40 days
Non treated	00	00	364	112	78
0.4ml	1	2	52	12	7
0.8ml	2	3	12	3	1
1.2ml	2	4	7	00	00
1.6ml	5	6	7	00	00

Table 2: Shown treatment of removal of *E. globules greeneries* in Aceton on the multiplicative activity of the *Callosobruchus maculates in* stored grains-*Vigna radata*.

Dosage in ml/kg	Death against exposure		Number of eggs placed	Infected grains	Emergence of Adults
	After 24hr	After 96hr	Till death of adults	Till death of adults	Up to 40 days
Non treated	00	00	400	180	123
0.4	2	4	121	50	23
0.8	2	3	86	25	9
1.2	2	3	25	13	5
1.6	5	4	20	6	00

Table 3: shown treatment of removal of *Eucalyptus globules greeneries* in aqueous phase on the multiplicative activity of the *C. maculates in* stored grains-*V. radata*.

Dosage in ml/kg	Death against exposure		Number of eggs placed	Infected grains	Emergence of Adults
	After 24hr	After 96hr	Till death of adults	Till death of adults	Up to 40 days
Non-treated	00	00	420	224	196
0.4	00	00	163	123	103
0.8	00	00	123	107	78
1.2	1	00	88	67	53
1.6	1	2	43	38	23

Table 4: *Effect of exposure of removal of Eucalyptus globulus greeneries in chloroform solvent on the reproductive activity C. maculates of the stored grains, V. aconitifolia.*

Dosage in ml/kg of grains	Death against exposure		Number of eggs placed	Infected grains	Emergence of Adults
	24hr	96hr	Till death of adults	Till death of adults	Up to 40 days
Non treated	00	00	560	214	140
0.4	1	2	137	64	31
0.8	2	3	83	41	22
1.2	3	5	57	13	00
1.6	5	7	81	7	00

Table 5: *Effect of exposure of removal of Eucalyptus globulus greeneries in acetone solvent on the reproductive activity C. maculates of the stored grains, V. aconitifolia.*

Dosage in ml/kg of grains	Death against exposure		Number of eggs placed	Infected grains	Emergence of Adults
	24hr	96hr	Till death of adults	Till death of adults	Up to 40 days
Non treated	00	00	406	143	142
0.4	1	2	146	72	35
0.8	2	5	124	65	27
1.2	2	6	98	32	4
1.6	4	3	24	00	00

Table 6: *Effect of exposure of removal of Eucalyptus globulus greeneries in aqueous solvent on the reproductive activity C. maculates of the stored grains, V. aconitifolia*

Dosage in ml/kg of grains	Death against exposure		Number of eggs laid	Infected grains	Emergence of Adults
	24hr	96hr	Till death of adults	Till death of adults	Up to 40 days
Non treated	00	00	512	185	200
0.4	00	00	159	82	71
0.8	00	00	132	74	69
1.2	1	2	128	64	53
1.6	1	2	99	42	37

Table 7: Result of exposure of extract of E. globules in acetone solvent on the weight and protein loss in grains of Phaseolus radiatus by the stored grain pest, C. maculates

Dose (ml extract/ Kg Grains)	Initial- weight of grain-s (gm)	Total numb-er of grains	Num-ber of Infected grains in 40 days	Wt. of 100 solid grains (gm)	Wt. of 100 hollow grains	% Wt. Loss in infect-ed grains	% Wt. Los-s in total grains	% Protein contents of solid grains	% Protein contents of hollow grains	% Protei-n loss in infected grains	% Prote-in loss in total grains
(A)	(B)		(C)	(D)	(E)	(F)	(G)	(H)	(I)	(J)	(K)
Control	25	512+ 32	112	4.995±0.11	0.560±0.14	88.78	20.69	27.54±1.30	9.40±0.60	65.86	20.69
0.4	24	512±32	12	4.995±0.11	2.754±0.17	44.86	1.12	27.54±1.30	17.68±1.02	35.80	1.120
0.8	24	512±32	3	4.995±0.11	3.968±0.24	20.56	0.128	27.54±1.30	21.36±0.11	22.44	0.128
1.2	24	512±32	Nil	4.995±0.11	-	-	-	27.54±1.30	-	-	-
1.6	24	512±32	Nil	4.995±0.11	-	-	-	27.54±1.30	-	-	-

± indicates S. D. of three observations

Table 8: Effect of chloroform extract of *Eucalyptus globules* leaves on the weight and protein loss in grains of *V. aconitifolia* by the stored grain pest, *C. maculates.*

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)
Con-trol	25	1084 ±42	143	2.311±0.12	0.326± 0.18	85.89	11.83	23.82±1.30	9.73±0.62	59.15	11.83
0.4	24	1085±42	72	2.312±0.12	1.23±0.02	46.79	3.24	23.82±1.30	16.58±1.20	30.39	3.24
0.8	24	1085±42	65	2.312±0.12	1.16±0.01	49.82	3.12	23.82±1.30	18.46±1.06	22.50	3.12
1.2	24	1085±42	32	2.312±0.12	1.06±0.12	54.15	1.66	23.82±1.30	21.22±1.46	10.91	1.66
1.6	24	1085±42	00	2.312±0.12				23.82±1.30			

± indicates S. D. of three observations.

Eucalyptus globules plant with greeneries (Plate I, fig. a) were used for makeup of extracts for the powder in different solvent system. Plate II shown the experimental set up and the plate III were shown variable number of eggs of *C. maculates* pasted to the grains. The grains of *V. aconitifolia and V. radiata* earlier releasing the *C. maculatus* males and females and after 40 days in control set are given in the plate III which shows hollow in appearance.

Photo Plate- I (Fig. a to Fig. e): Selected materials for experimental setups shown as *Eucalyptus globules* in fig. 1; Fig a and Fig. c were shown as *V. aconitifolia and V. radiata* before the treatments of experiments; Fig. d shown as a Soxhlet Apparatus for extraction of extract of leaves of *Eucalyptus globules* and in Fig. 5 shown the species of *C. maculates* which heavily attacks to common pulses of Vigna *aconitifolia and Vigna radiata. V. aconitifolia and V. radiata* food grains, if remains for few months in the household are suspectable to pests and makes them inadequate for eating purpose. Photo Plate- II (Fig. f to i): Investigational setup in the research. *C. maculates* arises usually in most of the pulses (as in Plate II, fig. h & i). The number of eggs attached on single grain varies from one to five (Plate II). The female laid eggs within 8 to 10 days. Photo Plate- III (Fig. j to m): Controlled (non-exposed) and treated (exposed) to *C. maculates* shown plague parameters of grains. *C. maculates* eat the grains from inside and were makes them muffled and then pupates as shown in Plate III, fig k and Fig m. The pupa can be detached from the grain. After about 28 days, the adult appears out from the grains. The Result of extracts of leaves plants, *Eucalyptus globules* with different solvents were presented variable damages and data were collected and tabularized in the form of different tables as from 1.1 to 1.6 and from 2.1 to 2.2. Due to the action of formulated bio-pesticide different stages of life cycle of *C. maculatus* was adversely affected. Egg forming, Eggs to larva and adult formation were affected as concentration of said pesticide were increased as par shown in tables. In control of *V. radiata*, from 364 eggs laid only 112 has been verminous the grains, and from 112 only 78 adults appeared which is shown in Table 1.1. In control of *Vigna aconitifolia*, from available of 406 eggs laid only 143 has crawling the grains, and from 143 only 106 adults occurred as shownTable 1.5.

Tables 1.1 to 1.3 shows the effect of extracts of *leaves of Eucalyptus globulus in* chloroform, acetone, and aqueous on the death rate of adult *C. maculatus* and their different stages of life cycle in the *Vigna radiata grains*. Although on other hand, Tables 1.4 to 1.6 shows the effect extracts of *leaves of Eucalyptus globulus* in chloroform, acetone, and aqueous on the death of adult *Callosobruchus maculatus* and their different stages of life cycle in the *Vigna aconitifolia grains*. Greeneries extracts in aqueous form result were shown as in Tables 1.3 and 1.6. It was observed deprived toxic effects and poor inhibitory effect on the generative activity.

Tables 2.1 to 2.2. were shown the injury caused by the *Callosobruchus maculates* in the control and the treated grains of *Vigna aconitifolia and Vigna radiata.* Amount of loss in the weight of the infested, *Vigna radiata by Callosobruchus maculatus* was 88.78 and in the total grains was 20.69 during the experiment period of 40 days in the nontreated (Control) grains (Table2.1). While amount of loss in the weight of the infested pulses of *Vigna aconitifolia* by *Callosobruchus maculatus* was 85.89 and in the total grains was 11.83 during the experiment period of 40 days in the nontreated (Control) grains (Table 2.2). Weight loss in infected and total grains was condensed in low concentrations of the extract of *Eucalyptus globulus* in chloroform *for Vigna radiata. Loss was* was nil at and above 1.2 ml per Kg. extract. The entire grains of *Vigna radiata* were found to have 27.54 % proteins while the infected hollow grains had only 17.68 and 21.36 % proteins at exposure of t 0.4- and 0.8-ml chloroform extract of leaves of *Eucalyptus globulus respectively.* Amount of Protein loss was minimized in different attentions of acetone extracts but the impact was deprived with respect to the aqueous extracts of *Eucalyptus globulus* as in the tables 2.1. Out of a hundred protin weight loss in infected and total grains was minimized in low concentrations of the chloroform extract of *Eucalyptus globules. for Vigna aconitifolia also* and was nil in concentrations above 1.6 ml per Kg. extract.

The entire grains of *Vigna aconitifolia* were found to have 23.82 % proteins while the infected hollow grains had only 16.58, 18.46 and 21.22 % proteins on exposure to 0.4, 0.8- and 1.2-ml chloroform extract of leaves of *Eucalyptus globulus* while percent protein loss in total grains at the said concentrations was only 3.24, 3.12 and 1.66. Protein loss was also minimalized in different concentrations of acetone extracts but the impact was poor.

Discussion

Any normal compounds of plant origin shown biodegradable properties, often of low mammalian poisonousness, and stance low danger to the environment. Present scenario of research has focused on natural product as alternatives for pest control. Various studies have demonstrated the effectiveness of neem (*Azadirachta indica* A. Juss.: Meliaceae) as a protectant [8, 9, 10].

Accourding to [11], seed beetles (e.g., *C. chinensis, C. maculatus, C. phaseoli, and Zabrotes subfasciatus)* have been widely used as the experimental organisms in research studies. Density-dependent egg death has been observed not only in *C. chinensis*, but also in other seed beetles. Reported that the oviposition marker of *C. maculatus* is very similar to that of *C. chinensis* [12]. studied the effect of neem oil along with three other non-edible oils against *C. chinensis.* It was found that the growth index with neem was the lowest; neem also had the greatest ovicidal effect. As par studies of [13], the effects of chickpea storage duration on oviposition of *C. chinensis* when the seeds were treated with neem oil. 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 [14].

assessed toxicity of rapeseed, *Brassica napus* extracts in the laboratory for toxicity, reduction of F-l progeny and repellency effects against adults of *Sitophilus oryzae* (L.) and *Rhizopertha dominica* (F.) at different concentrations; whereas data showed that surface treatment of wheat seeds with acetone or petroleum ether extracts of B. *napus* proved to be the most efficient extracts as they gave 51.8 % mortality among *S. oryzae* adults at 4.0% conc. Level [5]. reported that the extracts of *Anonna squamosa* were highly operative as antifeedant and as growth regulators [15] reported that a petroleum ether extract of neem leaves and twigs mixed with green gram seeds suppressed the oviposition of *C. chinensis* [16]. has shown that expressed extract of *Syzygigum cumini* marks to *Callosobruchus macultus.*

Conclusion

From the present research study it was concluded that for the effect of extract of greeneries of *Eucalyptus globulus* were shown effectiveness against growth of *Callosobruchus macultus.* Through observations and result it was shown that extract leaves of *Eucalyptus globulus* is adversely effective against growth parameters of *C. macultus* in different solvent systems at different concentrations. The nutritive values of food grains were reserved by saving from the loss of protein amount. As the formulated or screened pesticide is plant origin, it has no any adverse effect to humans and environment.

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