"A study of insecticidal effects of leaf extracts of *Aegle marmelos*(L.) and *Ricinus communis*(L.) on *Callosobruchus chinensis*(L.)"

Abstract:-

Laboratory experiments were conducted to evaluate the efficiency of leaf powders of *Aegle marmelos* and *Ricinus communis* against the pulse beetle, *Callosobruchus chinensis.* which infests stored pulse seeds. Pulses specially stored for the purpose of human consumption should not be treated with toxic chemicals because residues of these chemical compounds may pose a serious risk to human health. Stored pulses are primarily attacks by major insect pest particularly the pulse beetle, *Callosobruchus chinensis.* Though chemicals provide effective control of *Callosobruchus* sp., but the efficiency of the chemicals mostly depends on the mode of exposure. The maximum effectiveness is given when the insecticides are provided by gustatory method, but majority of these are toxic to human. Many synthetic insecticides have been found effective against stored product pests but proved to be hazardous to men and domestic animals. The over reliance on and non judicious use of synthetic pesticides especially insecticides since last four decades led to wide spectrum of pests problem like pests resistance to chemicals, resurgence of pests, residues in food and soil and risks to human and animal health, besides environmental pollution. The methanol and chloroform fraction of *Aegle marmelos* and *Ricinus communis* proved effective for the control of *Callosobruchus chinensis*. The findings suggest that plant extracted bio-pesticides may play a major role in insect pests control program in near future.

Key words:-

Aegle marmelos and *Ricinus communis,* leaf powders, *Callosobruchus chinensis,* pulse protection, biopesticides .

Introduction:-

Pulses are an important source of nutrition for billions of people around the world. The terms "legumes" and "pulses" are interchangeable because all pulses are considered legumes but not all legumes are considered pulses. Pulses belong to leguminoseae family and include those species that are consumed by human beings and domestic animals, commonly in the form of dry grains and does not include groundnut (*Arachishypogaea*) and soybean (*Glycine max*) which are grown mainly for edible oil. The Food and Agriculture Organization of the United Nations has declared 2016 as the International Year of Pulses with aims to enhance public awareness of the nutritional benefits of pulses as part of sustainable food production. This was intended To wards global food security and nutrition.

Pulses are rich source of many vitamins and minerals (iron, zinc, calcium, magnesium). The deficiency of various minerals in different parts of the world led to cardiovascular disease and imbalance in majority of the biological pathways. Pulses can provide adequate minerals required to fulfill nutritional requirement. Phenolic compounds are grouped into phenolic acids, tannins and flavonoids. Phenolic compounds and antioxidant activity also varied among different varieties of pulses.

Pulses are also being used by the processors worldwide for developing canned products as they are ready to use and the demand is expected to increase as they are having high shelf life (**Warsame and Kimani 2014).** Varieties with ease of preparation, processing efficiency and high yield of raw product were mostly preferred by the processors for thermal processing (**Wassimi et al. 1990; Hosfield et al. 2000).**

Pulse varieties with uniform size and rapid expansion during soaking, high water holding capacity during processing and less splitting are required to be developed for canning. The effects of conventional processing methods (soaking, dehulling, boiling, pressure cooking, germination, fermentation etc.) on the levels of anti-nutritional factors (phytate, protein inhibitors, phenolics, tannins, lectins, saponins etc.) have been extensively evaluated, however, novel methods to eliminate these needs to be developed (**Patterson et al. 2017).** Canning of pulses also reported to decrease phenolic content (**Parmar et al. 2016).**

Though the pulses are used in number of indigenous products but their functional attributes and potential health benefits have not been fully explored.

Pulses play a significant role in a country like India, and serve as an important part of the diet. They are considered to be as the “poor man’s meat” given that the consumption of dairy and animal products is relatively low among the poorest section of the country. They are also an important source of protein, minerals and fibre. The protein content in pulses is double the protein content of wheat, and three times more than that of rice. Additionally, pulses are used as green manure and contribute in improving soil health, offering the possibility for a mixed/ intercropping system (**Rawal and Navarro, 2019).**

Pulses as a commodity group fit in all the Feed the Future Initiative themes aiming toward sustainable poverty and hunger reduction and enhancement of nutrition and health conditions alongside protection of the environment (**Maredia, 2012).**

Major pulses are grown chickpeas (Gram/Chana), Pigeon pea (Tur/Arhar), Mungbeans, Urdbeans (Blackgram), Masur (Lentil), Peas and various kinds of Beans (Minor Pulses). · The main regions with high productivity are Madhya Pradesh, Rajasthan, Maharashtra, Karnataka, Uttar Pradesh, Coastal Andhra Pradesh,Gujarat, Tamil Nadu, Jharkhand, Odisha, Chhattisgarh, Telangana, Bihar and West Bengal delta region.

The census projection report has further revealed that the proportion of the working age population between 15 and 59 years is likely to increase from 58% in 2001 to > 64 per cent by 2021. Such a trend would make the country one of the youngest nations in the world. Thus, one of the India's competitive advantages is its demographic dividend.

Despite the imports, in 2019, the consumption of pulses in India amounted to 48 g per capita per day, slightly less than the 50 g per capita per day recommendation of the Indian Medical Research Council. One of the major hurdles in meeting self-sufficiency in pulses is policies that promote staple crop production, such as subsidies for fertilizers and credit and irrigation facilities that discourage the production of pulses and other legumes (**FAO et al., 2020).**

Material and Methods:-

Insect (*Callosobruchus chinensis)-*

Callosobruchus chinensis is major pest of pulses in India .It is a holometabolic insect with the egg and adult stage found on grains and the pupal stages living inside the grain.

As the larva eats up the endosperm of in most damaging stage of the life cycle. Adult beetles are 3-4mm in length, oval in shape, chocolate / reddish brown in colour, and have long erected antennae.

The female lays between 1-8 oval shaped and scale like egg grain. each larva completes the life cycle in a separate chamber.

In India the insect breeds feely from March to November and hibernates in the larval stage during the winter. The adult emergence takes place from January to April. The pest causes maximum damage during February to August, when all its developmental stages exist simultaneously.

Selection of plant-

Aegle marmelos and *Ricinus communis* was selected for the study because of its insecticidal and medicinal properties, and its abundance in the study area.

Collection of plant material-

The indigenous plant *Aegle marmelos* leaves of family Rutaceae and plant *Ricinus communis* leaves of family Euphorbiaceae was collected from the roadside of laharpur Bhopal, collection was done in winter season.

Taxonomic position of the plant-

Identification of the collected plant was carried out in the Department of Botany of the research center.

***Aegle Marmelos*(Bael)-**

Kingdom - Plantae

Order -Sopindales

Family - Rutaceae

Sub family – Aurantioideae

Genus -*Aegle*

Species - *marmelos*

The biological and pharmacological activity against various chronic diseases such as cancer and cardio vascular and gastrointestinal disorders.

Antioxidant, antiulcer, antidiabetic, anticancer, antihyperlipidaemic, anti inflammatory, antimicrobial, antispermatogenic effects have also been reported on various animal models by the crude extracts of this plant. Every part of *Aegle marmelos.*

Bael leaves could also be used as a potential biosorbant. noxious lead ions were demonstrated to be removed from an aqueous solution by absorbing them into the bael leaves. An unusual fatty acid, 12-hydroxyoctadec-cis-9-enoic acid (ricinoleic acid), is present in the bael seed oil, which has the potential to be manufactured as biodiesel in the future. structure of bael exudate gum was also determine which could be further characterized for various industrial applications. (**Suvimol et al., 2008).**

***Ricinus communis* (castor):-**

Kingdom - Plantae

Division -Magnoliophyta

Class -Magnoliopsida

Subclass -Rosidae

Order -Euphorbiales

Family - Euphorbiaceae

Genus -*Ricinus*

Species - *communis*

The leaves in applied to boils and swelling the hot leaves are applied over the abdomen of children to relieve flatulence. The leaves promote menstrual flow in women. Tender leaves are cure pain in bladder, leaves are also recommended to relieve headache and joints pains.(**Jena J and Gupta, 2012.)**

For phytochemical analysis of plant the collected material after identification was used and a voucher specimen was procured in the herbarium sheet. The shade dried and powdered leaves were soxhlated in 90% alcohol, rectified spirit and water respectively.

The extract thus obtained was kept in a glass vial and stored in the refrigerator; percentage yield was recorded in the tabular form (Table No.1). Percentage loss in weight after drying of the plant material was also recorded which showed 96.2% loss in weight.

The cold percolation method as mentioned by **Abbott (1925**) was followed for fresh plant leaves. The air dried plant material was powdered using electrical blender. 500 gm of powder was mixed with 300ml of n-hexane and kept for 72 hrs then filtered and it was stored in a reagent bottle. The powder was allowed to dry for 2 hrs before pouring the other solvents-methanol and chloroform and kept for 72 hrs. The crude extracts thus obtained were filtered through Whatman's filter paper No. 1 and were evaporated to dryness in a rotary vacuum evaporator or water bath at the room temperature (400C) and pressure (25-30mm hg). Such dried and semisolid crude extract was stored in refrigerator the use i.e for bioassay test against *C. chinensis.*

Chemical analysis and identification of the compounds

First the crude extract of plant was defatted in n-hexane and extracted with methanol and chloroform. The concentrated solution was allowed to stand when a green yellow deposit was obtained.

For further identification and structural elucidation of plant extract, the purified sample was sent to SAIF, CDRI Lucknow for spectral analysis: where IR spectrum, UV spectrum, NMR and Mass spectrum were done.

On the basis of spectral data obtained from SAIF CDRI Lucknow and on comparing the data with authentic markers available finally, sesquiterpene lactone was identified.

Study of *Aegle marmelos* and *Ricinus communis* leaf extract on *Callosobruchus chinensis*

The collection of insects was done from the infested grains.

20 beakers were taken. They were washed with distilled water and then dried. In each beaker 100 seeds of pulse (e.g. Uradbean) were taken.

In each beaker two drops of chemical: methanol and chloroform fractions of different concentrations e. g. 50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm were poured.

In each beaker 3 pairs of insects (3 males and 3 females) were introduced. The mouth of each beaker was covered with muslin cloth and tied with a rubber band and a control was kept.

After 7 days, the beakers were opened by removing the muslin cloth and the insects were taken out.

The eggs laid on the grains were counted and the beakers were covered with muslin cloths as before.

After 7 days, the beakers were opened for calculating the number of larva emerged from the eggs and then beakers were once again closed as before.

After another 7 days, once again the beakers were opened to calculate the number of pupae. Then beakers were once again closed with the muslin cloth.

After 7 days, again the beakers were opened to see the adult insects emerged from the pupae. They are counted.

The seeds of each beaker were taken in a petri dish and were wetted with water to observe their germination.

The seeds which germinated were counted.

The same experiment was repeated with the wrinkled seeds.

Table No.**-1**

Percentage loss in weight of the plants material

S. No.	Name of the plant	Wet weight of plant material in (gms)	Weight on drying of the plant material (gms)	Loss in weight on drying (gms)	Percentage loss in weight
1.	*Aegle marmelos* (Linn)	2000	180	1920	96.2%
2.	*Ricinus communis*(Linn)	2000	190	1932	98.4%

Result and Discussion:-

Hemantha piris et al. (2021) reported highly significant repellency 96.91% and 98.99% of dried leaf and seed kernel extract of *C. thevetia* and *O. tenuiflorum* against red flour beetle after 24 hour of exposure which indicates that botanicals have high repellency properties. **Likewise Gitahi et al. (2021).**

According to **Batish et al. 2008, Nerio et al. 2010.** Several plant extracts have been well documented by researchers for both their insect repellent and insecticide effects. A study by **Koona et al. (2007)** showed that the use of impregnated jute bags with *Chenopodium ambrosioides* and aqueous extracts from Lantana camara significantly reduced damage to stored pulses of *Acanthoscelides obtectus* (Say) and *C. maculatus*. Those locally available plant extracts can be used as an inexpensive source of insecticide.

In the present investigation the repellent activity of *Aegle marmelos* leaf extract in five concentrations against *C. chinensis* at one hour interval showed maximum repellent activity (90%) in 250ppm conc. of methanol and chloroform and minimum in control.

In the present investigation the repellent activity of *Ricinus communis* leaf extract in five concentrations against *C. chinensis* at one hour interval showed maximum repellent activity (90%) in 250ppm conc. of methanol and chloroform and minimum in control.

Aliyu and Ahmed (2006) reported that all non-edible oils caused larval and adult mortality to 100%, and also had ovipositional deterrence. The bruchids are controlled by oils extracted from some plant mater revealed that groundnut oil and *Mentha arvensis* (L), *M. spicata* (L), *M. piperata* (L) and *Cymbopogon nardus* (L) have an effect on bruchid.

In the present study of research work larval mortality was observed maximum in 250 ppm conc. Pf chloroform and methanol extract of *Aegle marmelos* and *Ricinus communis* leaf while it was minimumin the control.

Chakraborty et al., 2004 Based on total life cycle of *C. chinensis* on various pulses, the order of pulses were chickpea >black gram > greengram >. However, it is evident from the literature that higher seed weight and thick seed coat prolonged developmental period In spite of higher nutrition, the development is retarded because of thick seed coat which creates obstruction for penetration of young larvae inside the seed and emergence of adult from the seed.

In the present study the leaf extract of experimental plant inhinited adult emergence. Minimum adults emerged in methanaol and chloroform 250 ppm conc.& minimum in the control.

Table No.-2

Statistical data of purified fraction of *Aegle marmelos* (AMF$_5$) methanol extract against Pulse beetle *(Collosobruchus chinensis)* on fresh Urad bean seeds.

Concentration	**24 hr. larval mortality**	**Regression equation (y = a±bx)**	**Chi - Square x^2(n-1)**	**LC$_{50}$ (ppm)**	**Variance (V)**	**S.E.**	**Fiducial limits (ppm)**
50	68	-3.669±1.773 x	6.347	117.372	0.0243	0.156	L = 89.224 U = 147.830
100	92						
150	145						
200	158						
250	175						

Table No.-3

Statistical data of purified fraction of *Aegle marmelos* (ACF_5) Chloroform extract against Pulse beetle *(Collosobruchus chinensis)* on fresh Urad bean seeds.

Concentration	24 hr. larval mortality	Regression equation (y = a±bx)	Chi - Square x^2(n-1)	LC_{50} (ppm)	Variance (V)	S.E.	Fiducial limits (ppm)
50	73	-3.775±1.832 x	20.703	115.082	0.0243	0.156	L = 56.150 U = 183.278
100	99						
150	119						
200	159						
250	195						
Control	6						

Table No.-4

Statistical data of purified fraction of *Aegle marmelos* (AMF_5) methanol extract against Pulse beetle *(Collosobruchus chinensis)* on Wrinkled Urad bean seeds.

Concentration	24 hr. larval mortality	Regrassion equation (y = a±bx)	Chi - Square x^2(n-1)	LC_{50} (ppm)	Variance (V)	S.E.	Fiducial limits (ppm)
50	79	-3.82±1.922 x	9.606	98.665	0.0246	0.157	L = 66.191 U = 127.892
100	104						
150	149						
200	173						
250	197						
Control	6						

Table No-5

Statistical data of purified fraction of *Aegle marmelos* (ACF_5) Chloroform extract against Pulse beetle *(Collosobruchus chinensis)* on Wrinkled Urad bean seeds.

Concentration	24 hr. larval mortality	Regression equation (y = a±bx)	Chi - Square x^2(n-1)	LC_{50} (ppm)	Variance (V)	S.E.	Fiducial limits (ppm)
50	71	-4.262±2.109 x	11.656	104.906	0.0252	0.159	L = 71.848 U = 136.552
100	99						
150	142						
200	176						
250	199						
Control	6						

Table No-6

Statistical data of purified fraction of *Ricinus Communis* (RMF_5) methanol extract against Pulse beetle *(Collosobruchus chinensis)* on fresh Gram/chickpea seeds.

Concentration	24 hr. larval mortality	Regression equation (y = a±bx)	Chi - Square x^2(n-1)	LC_{50} (ppm)	Variance (V)	S.E.	Fiducial limits (ppm)
50	79	-3.873±1.929 x	14.774	101.805	0.0246	0.157	L = 58.895 U = 140.919
100	99						
150	144						
200	170						
250	199						
Control	9						

Table No-7

Statistical data of purified fraction of *Ricinus Communis* (RCF_5) Chloroform extract against Pulse beetle *(Collosobruchus chinensis)* on fresh Gram/chickpea seeds.

Concentration	24 hr. larval mortality	Regression equation (y = a±bx)	Chi - Square x^2(n-1)	LC_{50} (ppm)	Variance (V)	S.E.	Fiducial limits (ppm)
50	60	-5.018±2.416 x	32.378	119.360	0.02689	0.164	L = 62.022 U = 190.732
100	79						
150	128						
200	166						
250	209						
Control	9						

Table No.-8

Statistical data of purified fraction of *Ricinus Communis* (RMF_5) methanol extract against Pulse beetle *(Collosobruchus chinensis)* on Wrinkled Gram/chickpea seeds.

Concentration	24 hr. larval mortality	Regression equation (y = a±bx)	Chi - Square x^2(n-1)	LC_{50} (ppm)	Variance (V)	S.E.	Fiducial limits (ppm)
50	89	-4.317±2.224 x	35.106	87.411	0.0259	0.161	L = 18.279 U = 137.155
100	110						
150	149						
200	189						
250	223						
Control	9						

Table No.-9

Statistical data of purified fraction of *Ricinus Communis* (RCF_5) Chloroform extract against Pulse beetle *(Collosobruchus chinensis)* on Wrinkled Gram/chickpea seeds.

Concentration	24 hr. larval mortality	Regression equation (y = a±bx)	Chi - Square x^2(n-1)	LC_{50} (ppm)	Variance (V)	S.E.	Fiducial limits (ppm)
50	63	-4.880±2.386 x	30.443	111.045	0.0268	0.164	L = 55.138 U = 169.725
100	94						
150	134						
200	165						
250	216						
Control	9						

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