Relative efficacy of certain new molecules and botanicals as seed protectant on storability of green gram and black gram against pulse beetle, *Callosobruchus chinensis* L.

A Thesis submitted to the
Orissa University of Agriculture and Technology
in partial fulfilment of the requirement for the degree of
Master of Science in Agriculture
(Seed Science and Technology)

By

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BHUBANESWAR- 751003, ODISHA
2015

ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
ALL INDIA COORDINATED RESEARCH PROJECT ON STR
COLLEGE OF AGRICULTURE
BHUBANESWAR-751003, ODISHA

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CERTIFICATE - I

This is to certify that the thesis entitled **"Relative efficacy of certain new molecules and botanicals as seed protectant on storability of green gram and black gram against pulse beetle, *Callosobruchus chinensis* L."** submitted in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE IN AGRICULTURE (SEED SCIENCE AND TECHNOLOGY)** of the **ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY**, Bhubaneswar is a faithful record of *bona fide* research work carried out by **NAMASHYA MISHRA** under my guidance and supervision. No part of the thesis has been submitted for the award of any other degree or diploma.

It is further certified that the assistance and help availed by him from various sources during the course of investigation has been duly acknowledged.

Chairman
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CERTIFICATE-II

This is to certify that the thesis entitled **"Relative efficacy of certain new molecules and botanicals as seed protectant on storability of green gram and black gram against pulse beetle, *Callosobruchus chinensis* L."** submitted by **NAMASHYA MISHRA** to the Odisha University of Agriculture and Technology, Bhubaneswar in partial fulfillment of the requirements for the degree of **MASTERS OF SCIENCE IN AGRICULTURE (SEED SCIENCE AND TECHNOLOGY)** has been approved by the students' advisory committee and external examiner.

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ACKNOWLEDGEMENT

"We are what we repeatedly do.
Excellence, therefore, is not an act but a habit."

"To the sovereign God, I give all the glory". Emotions cannot be adequately expressed in words. Because then, emotions are transferred into mere formality and formalities have to be completed. Acknowledgement in true essence, gives us an opportunity to remember and express our feelings for those whom we have and revere. When going gets tough and tougher, the helping hand offered then by all the near and dear ones is always remembered with gratitude.

I would like to give my first thanks to Almighty GOD and my PARENTS, as without their blessings, accomplishment of my work and preparation of this manuscript would have not been possible.

I have highest reverence towards my esteemed advisor Dr. D. Dash, A.S.R.O. (Entomology), AICRP on STR, Department of Seed Science and Technology for his magnanimous guidance, valuable suggestions, unending zeal, untired cooperation, constructive suggestions and constant support throughout the course of investigation and preparation of this manuscript.

I am extremely grateful to Dr. S.K. Mohanty, Professor and Head of the Department, Seed Science and Technology, College of Agriculture, O.U.A.T. Bhubaneswar and Chairman of advisory committee for providing the required facilities for enabling successful completion of the thesis work.

I avail the auspicious opportunity of expressing my deep sense of honour and gratitude to Dr. B.K. Mishra, Associate Professor and Head, R. Pattanaik, Associate Prof., P. Pattanaik, Department of Plant Physiology, College of Agriculture, O.U.A.T. Bhubaneswar, for his help rendered to complete my thesis work.

I also wish to express my sincere thanks and deep sense of appreciation to Dr. K.C. Muduli, Dr. B.B. Patro, Dr. A.K. Kar, Dr. S. K. Swain and Anita Priyadarshini Teachers of the Department of Seed Science and Technology for their timely cooperation, suggestions during the course of my study.

I convey my eternal thankfulness to Dr. B. Baisakh, Professor and Head of the Department, plant Breeding and Genetics, and Dr. P. Mishra, Associate Professor, Department of Entomology, College of Agriculture, O.U.A.T Bhubaneswar for his benevolent support, inspiring suggestion, positive criticism and benign facilities provided by him during the course of investigation

I feel it proper to express my deepest sense of thank to those authors and magazines, journals, books, handouts, research papers that I have referred to give this work to right direction.

I also have a special reverence to Jayanta Beura for his generous help to conduct the laboratory works .

At this juncture of time my heart is full, mouth is dump and I feel short of words at my command to express my regards and respect to my beloved parents Mr. Subash Chandra Mishra and Mrs. Madhuri Mishra.

It is neither possible nor probably within the scope of this "acknowledgement" to record any feeling of affection, love and thanks towards my batch mates. I am thankful to them for their support, encouragement and unrestrained help during period of this study.

The pleasant company, cooperation and melted out by help all my respected worthy seniors and all my loving juniors acknowledge with great regards.

Last but not least, I express my sincere thanks to all beloved, respected people who helped me directly or indirectly but could not find a separate mention.

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ABSTRACT

Studies were conducted in the Seed Entomology Laboratory of Department of Seed Science and Technology during 2014-15 to evaluate the efficacy of few insecticide molecules and botanicals as their seed treatment in green gram and black gram against pulse beetle, *Callosobruchus chinensis* (L.) infestation under ambient storage condition. The insecticidal and botanical experiments were laid out in CRD with 6 treatments including untreated control and replicated four times . Test insecticides molecules comprised Emamectin benzoate 5 SG @ 2ppm, Spinosad 45 SC @ 2ppm, Profenofos 50 EC @ 2ppm, Novaluron 10 EC @ 5ppm and Deltamethrin 2.8 EC @ 1ppm. Different plant materials like begunia, naguari, curry leaf powder@ 10g/kg seed, citronella oil @ 10 ml and sweet flag formulation 6 EC @ 10 ml/ kg seed were taken as test botanicals for study.

Studies on bruchid mortality indicate no mortality in untreated control as against 45.0 to 77.5% and 45.0 to 75.0% mortality due to insecticidal and botanical treatments respectively following 7 days exposure period after 6 months of seed treatment irrespective of pulses. The seed treatment with deltamethrin (7.25 eggs) and emamectin benzoate (8.75 eggs) recorded minimum fecundity of pulse seeds in green gram and black gram respectively. Among botanicals significantly lowest oviposition was noticed in sweet flag formulation both in green gram (8.0 eggs) and black gram (8.5 eggs) after 6 months of storage.

The population build-up of adult bruchids(F_1progeny)was significantly minimum in emamectin benzoate (7.0- 8.25 adults) followed by deltamethrin (8.25- 9.75 adults) as against maximum in untreated control (75.50- 84.50 adults) regarded of pulses, after 6 months of seed treatment. On the contrary, the seed treatment with sweet flag formulation registered lowest adult emergence in green gram (2.75- 7.25 adults) and black gram (1.75-6.25 adults) through out the period of investigation.

Highest reduction of seed damage on control was estimated in emamectin benzoate (90.70%) and spinosad (88.00%) in green gram and black gram, respectively. Among plant products, the percentage reduction of seed infestation over control after 6 months of storage was recorded maximum in sweet flag formulation (82.69) in green gram and begunia leaf powder (86.96) in black gram.

Emamectin benzoate was found best and expressed highest germination count (85.50 -86.75 %) among insecticides in both the treated pulse seeds exposed to bruchid attack after 6 months of storage period. Treatment with sweet flag formulation contributed a maximum of 22.39 and 19.72 per cent increase in germination potential of green and black gram seeds respectively.

The weight loss and protein content of infested green gram seeds was calculated least in emamectin benzoate (0.22 and 13.44 %) followed by deltamethrin (0.24 and 13.75 %). In black gram, it was analysed minimum in emamectin benzoate (0.33 % and 12.96 % followed by spinosad (0.39 % and 13.90 %). Among botanicals,sweet flag formulation showed lowest weight loss and protein composition in green gram (0.38 % and 13.96 %) and black gram (0.35 and 13.25 %) due to minimum seed damage by bruchids.

Thus it can be concluded that new molecules i.e., emamectin benzoate, spinosad and botanicals i.e., sweet flag formulation, begunia leaf powder and citronella oil were found most effective seed protectants in pulses over others and have great potentiality in suppressing the seed infestation by the bruchid to a minimum level with appreciably no adverse effect on seed viability up to 6 months of storage.

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CHAPTER-I

INTRODUCTION

INTRODUCTION

Pulse crops play an important role in Indian agriculture not only through supplying protein rich food but also improve and enrich soil health through adding atmospheric nitrogen to soil. Pulses contain 20 to 30 per cent protein of which lysine is of great importance and serves as the best means of solving malnutrition problems in the vegetarian Indian diet. Undoubtedly, the pulses have been considered as poor mans' meet for underprivileged people who can not afford animal protein. Keeping in view large benefits of pulses for human health the United Nation has proclaimed 2016 as the International Year of pulses.

Green gram is a native of India and central Asia. It is highly nutritious containing 24.6 % protein, 1.0 % fat, 57.5 % carbohydrate, Ca 0.08 g, P 0.045 g, Fe 5.7 mg, vitamin 750 IU thiamin 0.525 mg, riboflavin 300 mg, fibre 2.2 g per 100 g seed and provides 234 cal energy (Srivastava and Ali, 2004). It contains 3 times more protein than cereals. Maharashtra is the largest producer of green gram accounting 23.05 % of total production. Similarly black gram are highly nutritious containing 347 cal energy, 24 g protein, 3 g mineral, 1 g fibre, 60 g carbohydrates, 154 mg Ca, 4 g P per 100 g. Important states producing black gram are Maharashtra, UP, AP, Odisha, Tamil Nadu, Rajasthan, Chhatisgarh and MP. Pulses are grown more than 171 countries in the world. Pulses occupy 68.32 million ha area and contribute 57.51 million tonnes to world's food basket. India shares 35.2 per cent area and is the largest producer accounting 27.65 per cent of the global production of quality seeds (Chaturvedi and Ali, 2002). Seed is an essential input for crop production. Quality seed plays crucial role in deciding production potential and eventually contributes in better agricultural output in terms of both volume and value. India has achieved a record pulse production of 18.45 million tonnes in the year 2012-13 on 239 lakh ha area. Odisha has recorded the pulse production to the tune of 10.37 lakh metric tonnes on 24.42 lakh hectare area during 2012-13 with average yield of 508 kg/ha. Green gram is cultivated in an area about 8.83 lakh ha with total production of 3.96 lakh metric tonnes and average yield around 476 kg/ha. The total area under black gram cultivation comes around 5.92 lakh ha with production of 2.70 lakh metric tonnes and average yield of 457 kg/ha.

Pulses are generally stored for about a year at farmer, trader and government levels in various types of storage structures until the harvest of next crop. During the post harvest period, particularly in storage, a sizeable loss of pulses is observed in terms of quality and quantity. According to world bank report, post harvest losses in India accounts to 12-16 million metric tonnes of food grains each year, an amount that world bank stipulates could feed one-third of India's poor. The monetary value of these losses amounts to more than 50,000 crores per year (Singh, 2010).

Major constraints for the production and protection of pulses are insect pest infestation in field and storage. Among different insect pests attacking pulses, bruchids, *Callosobruchus* spp. are most notorious causing heavy damage and severe losses in storage. Of several species of pulse beetle, *Callosobruchus chinensis* (L.) is most wide spread and destructive major insect pest of economically important leguminous grains such as greengram, black gram, chick pea, peas, cowpea, lentil, pegion pea (Park *et al.*, 2003). The bruchids attack the pods of pulses in the field from where they are carried to godown and completely destroy the endosperm of grains causing loss in viability of seeds, unsuitable for human consumption, for sowing or for production of sprouts. Due to short life cycle and high degree of reproductive capacity, losses caused by this beetle to the pulses have been estimated to the tune of 40-50 % in storage (Shaya *et al.*,1997). It has been suggested that the growth and development of *Callosobruchus* spp. depends on nutritional value of seeds. The huge losses caused by bruchids remains the biggest impediment in the safe storage of pulses.

Success achieved making the stored products free from pests has been largely dependent on pesticides alone which are most powerful tool for pest control. The conventional management of *Callosobruchus* spp. mainly depends upon the use of synthetic pyrethroids and few organophosphates. In the past, a large number of synthetic pyrethroids have been tested and have received greater attention for controlling stored grain insect pests in India. Their continuous and discriminate use has led to the development of manifold resistance leading to frequent outbreaks of this pest. This has promoted the necessity for the development of new, safer and more potent insecticides that could provide feasible and effective insect pest management. Identification of potential insecticides which could be utilized for effective management of the pest is the need of the hour. Among different methods, the seed treatment with chemicals remain major choice. It is cheap, easy to apply and cost-effective. The prophylactic seed treatment with these insecticides is also very important to check the cross-infestation of stored grain insect pests. The effect of new molecules for their efficacy against the bruchids has not yet been studied so far except very few and rare cases.Information relating to this aspect is very scanty and not available in published literature.

Keeping in view a few benign insecticides with novel mode of action were bioassayed as seed treatment in green gram and black gram in order to identity the potential molecules for developing proper management strategy against the pest. Most of these new insecticidal molecules have been synthesized from naturally occurring organisms,selective in nature,required in very small quantity, low mamalian toxicity, relatively safer to parasitoids and predators of pest and has been found highly persistent toxicity against the pest without development of resistance.

Alternative methods of insect control utilizing botanical products are being used in many countries. It has been owing to the man's tendency to substitute pesticides for effective bio-evrionmental controls rather than restrict their use to emergent situations. Plant derived materials are more readily biodegradable, relatively specific in mode of action and easy to use. They are environmentally safe, less hazardous, less expensive and readily available.

Different botanical formulations have been reported time to time showing pronounced insecticidal activity, repellence to pest, oviposition deterrrency, adult emergence inhibition, ovicidal, larvicidal, pupaecidal activity and feeding deterrency based on their contact toxicity and fumigation effects. Some of the botanicals have also been practically proved efficacious to protect the food commodities from the bruchids during storage conditions. Such botanical formulations have shown their promise in integrated management of the pest as semiochemicals by showing behavior altering efficacy against the bruchids, thereby, reducing the induced pest resistance problem which is frequently reported twith synthetic pesticides. Hence, they may be recommended in food security programmes as eco-friendly and bio-rational alternatives of synthetic pesticides providing integrated management of the losses of stored food commodities due to infestation of bruchids (Kedia *et al.*, 2013).

Though, our country is very rich in flora of medicinal and other indigenous plants, yet not much works have been done to explore the possibilities of using them as grain/seed protectant under Indian conditions. From these points of view, it was felt necessary to orient efforts towards evaluation of some plant products as seed treatment for their bio efficacy against the bruchids in stored green gram and black gram.

Bruchids affects the stored legumes both qualitatively and quantitatively. Qualitative deterioration such as germination loss, increase in moisture, crude protein, total nitrogen & uric acid etc. are noticed in the bruchid infested pulses. The quantitative losses reduce the amount of food available per capita. Whereas the qualitative losses may necessarily affect the nutritional status of family. Attempt therefore, was made to determine quantitative and qualitative losses in pulse seeds due to bruchid infestation.

Keeping in mind the above aspects in view, the present investigation entitled "Relative efficacy of certain new molecules and botanicals as seed protectant on storability of green gram and black gram against pulse beetle, *Callosobruchus chinensis* L." was undertaken with following objectives.

(i) To evaluate the residual toxicity of chemical insecticides & botanicals on mortality of pulse beetles.

(ii) The study the biological parameters of pest on treated pulse seeds.

(iii) To study the effect of insecticides and botanicals on seed quality attributes.

(iv) To find out the most effective insecticide molecules and botanicals against the pest infestation in pulse seeds.

(v) To assess the quantitative losses and biochemical/nutritional changes on treated seeds due to bruchid infestation during storage.

CHAPTER-II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Under Indian conditions, more than 200 species of insects have been reported to infest different pulses (Dhaliwal and Arora,1994). Among different pulses pulse beetle(*Callosobruchus sp.*) is the most serious storage pest, the infestation of which starts in the field but its visual symptoms and secondary infestation inflicts heavy losses under storage conditions only (Rani and Mohan,2007).The damage is further accentuated due to failure of adoption of storage structures by farmers for storing pulse seeds. Thus the use of grain/seed protectants remain the major choice among farmers. Cost effective new synthetic insecticides having contact and low mammalian toxicity as prophylactic treatment remains the most feasible and played a significant role in managing the pulse beetle in stored seeds. The use of plant products as grain protectants is an age old practice and appears to be quite safe, promising and getting much emphasis in recent years(Jilani *et al.*,1988).Keeping this in view, the present study was undertaken to assess the efficacy of some new molecules and botanicals as seed protectants against the pulse beetle in stored green gram and black gram. The effect chemicals and botanicals on different parameters of test insect and test pulse seeds was studied during the period of investigation. The related/relevant references available in published literature pertaining to present study have been reviewed and described/narrated below.

2.1 Effect of insecticides on *Callosobruchus chinensis* and its impact on quality parameters in treated pulse seeds

Santhi *et al.* (1993) studied the effects of certain synthetic pyrethroids viz. decamethrin (40 and 20 ppm), cypermethrin (100 and 50 ppm), permethrin(100 and 50 ppm) and fenvalerate (100 and 50 ppm) on two important biological parameters viz. fecundity and adult emergence of pulse beetle, *C. maculatus* in comparision with popularly used insecticide malathion(40 and 20 ppm). Each gunny bag containing the seeds were surface treated and the insects were exposed to treated seeds. Cypermethrin and Deltamethrin suppressed adult emergence and oviposition of the beetle. At lower concentration the insecticides were effective for only up to 2 months while at higher concentration the effect persisted for up to 5 months. Comparatively higher fecundity and adult emeregence were noted in malathion.

Patil *et al.* (1994) studied the efficacy of seed treatment with five synthetic pyrethrois along with malathion against *C.maculatus* on pigeonpea seed at 4.8 and 12 weeks treatment. Deltamethrin (10, 12.5 ppm), fenvalerate (60, 75 ppm) and cyfloxylate (150ppm) were most effective treatments recording high adult mortality, lower egg laying, lower number of adults emerged and no loss in grain weight up to 12 weeks period whereas treatment with malathion (50 ppm) was not effective as it exhibited higher loss in grain weight at 4 weeks after treatment.None of the insecticidal treatment affected germination adversely.

Mohamed (1996) evaluated cotton stems ,cattle dung ashes and malathion as grain protectants against two species of bruchids. *Callosobruchus maculatus and C.chinensis* at various concentration. Results with mung bean(*Vigna radiata*) seeds showed that average mortalities of *C.maculatus* reached only 6.7 and 16.7% for cotton stems and cattle dung ashes, respectively and for *C.chinensis* 16.7 and 25.6% respectively for the same materials and dosage. Malathion showed very high efficiency of 94.4 and 93.3% for *C.maculatus* and *C.chinensis*, respectively. The corresponding mortalities of *C.maculatus* were 34.4 and 95.6%.

Narayan *et al.* (1997) reported that Fenvalerate 0.4 D and endosulfan 4D at 3 and 4 g ai/kg seed gave the most effective level of control of *C.chinensis* infesting green gram. Methyl parathion 2 D and quinalphos 1.5 D each at 2, 3 and 4 g ai/kg seed gave an inferior level of control compared to the recommended rate of malathion 5D @ 2.5 ai/kg seed. Insecticidal dust treated seeds had a lower infestation level of *C. chinensis* than untreated seeds.

Gupta *et al.* (1998) tested the effectiveness of insecticides,fungicides and storage containers against *C.chinensis* in mung bean seeds. Seed treatment with Deltamethrin @ 0.04ml/kg and thiram @ 2.5 g/kg gave complete protection under ambient conditions in gunny bags for 18 months without hampering viability.

Kalyan and Dadhich (1999) reported the malathion, cypermethrin and dichlorvos each at 5, 10, 15 ppm applied to seeds of *vigna radiata* provided 100% mortality of *C.maculatus* for up to 90 days after treatment. However, malathion and cypermthrin both at 10 and 15 ppm gave 100% adult mortality even upto 120 days, followed by dichlorvos 15 ppm (90%), malathion 5 ppm (80%), cypermethrin

5ppm(75.33%), dichlorvos 10 ppm (73.66%) and dichlorvos 5 ppm(68%). In untreated control, no pest mortality was observed at any of the observational intervals.

Narayan *et al.* (1999) studied the effect of insecticidal dust mixing and storage period on seed quality of green gram. Five insecticidal dusts viz. fervalerate 0.4D, methyl parathion 2 D, endosulphan 4D, quinalphos 1.5D each @ 2,3 and 4 g/kg seed along with recommended malathion 5 D @ 2.5 g/kg seed were mixed with green gram seed to evaluate the seed quality up to 180 days of storage. All the doses of fenvalerate 0.4 D, endosulfan 4D and malathion 5D improved germination whereas methyl parathion 2D and quinalphos 1.5D inhibited germination as compared to untreated control. As storage period of treated seeds increased the germination and vigour index decreased

Singh and Yadav (2001) investigated efficacy of four grain protectants against *Callosobruchus maculatus* Fab. on moth bean in laboratory conditions. Observations were taken at 0, 30, 60, 90,120,150, and 180 days interval. The grain protectants malathion (20 and 30 ppm) and cypermethrin(15 ppm) proved to be the highly effective, malathion (10ppm), cypermethrin(10ppm) and fenvalerate(15ppm) moderately effective and cypermethrin (5ppm), fenvalerate (5 and 10 ppm) and neem oil (4, 8 and 12 ml/kg grains) proved to be the least effective in causing mortality at all the intervals.

Abo-elghar *et al.* (2004) studied that three insect growth regulators (IGR), the chitin synthesis inhibitors (CSI) teflubenzuron and hexaflumuron and the juvenile hormone mimic (JHM) pyriproxyfen, as well as the organophosphate (OP) pirimiphos-methyl were evaluated for their activity against the cowpea weevil, *Callosobruchus maculatus* (F) in cowpea seeds stored for up to 8 months post-treatment. The initial activity data showed that, based on LC50 level, teflubenzuron had strong ovicidal activity (LC50 = 0.056 mg kg(-1)) followed by pirimiphos-methyl (1.82 mg kg(-1)) and pyriproxyfen (91.9 mg kg(-1)). The residual activity data showed that none of the IGRs tested had strong activity when applied at 200 mg kg(-1) in reducing the oviposition rates *of C maculatus* at various storage intervals up to 8 months post-treatment. However, teflubenzuron reduced adult emergence (F1 progeny), achieving control ranging from 96.2% at 1 month to 94.3% at 8 months. Hexaflumuron showed a similar trend in its residual activity, ranging between 93.8%

control at 1 month to 88.2% control at 8 months post-treatment. However, pyriproxyfen was more active than the CSIs tested and caused complete suppression (100% control) of adult emergence at all storage intervals. Unlike the IGRs tested, pirimiphos-methyl applied at 25 mg kg(-1) was more effective in reducing oviposition rates of *C maculatus* up to 8 months post-treatment. A strong reduction of adult emergence was also observed at various bimonthly intervals (98.6% control at 1 month to 91.6% control at 8 months post-treatment). The persistence of hexaflumuron and pirimiphos-methyl in cowpea seeds was also studied over a period of 8 months. The loss of hexaflumuron residue in treated cowpeas (200 mg kg(-1)) was very slow during the first month post-treatment (4.43%). At the end of 8 months, the residue level had declined significantly to 46.4% of the initial applied rate. The loss of pirimiphos-methyl residue in treated cowpeas (25 mg kg(-1)) was relatively high during the first month post-treatment (36.7%) and increased to 81.6% after 8 months.

Deshpande *et al.* (2004) evaluated the effect of seed treatment with plant products along with malathion dust against *Callosobruchus chinensis* (L.). The sweet flag rhizome powder @ 1.0 % followed by neem seed kernel powder @ 5 per cent exhibited superior effect against bruchid attack and also maintained the seed viability. Lakke leaf powder showed least effect during storage.

Srivastava and Jha (2007) evaluated the toxicity of commercial formulations of few synthetic pyrethriods viz., cypermethrin,bifenthrin,α-cypermethrin,β-cyfluthrin,α-cyhalothrin,fenvalerate and deltamethrin against three bruchid pests viz., *Callosobruchus chinensis, C maculatus and C analis.* All the tested species of *Callosobruchus spp.* showed similar order of toxicity for synthetic pyrethroids, deltamethrin being most toxic and fenvalerate the least. Bifenthrin and deltamethrin had similar toxicity against all the three species of pulse beetle. *C chinensis* was most susceptible against synthetic pyrethroids except cyfluthrin and fenvalerate which showed maximum toxicity against *C analis and C maculatus* respectively.

Laboratory and field trials were carried out to determine the efficacy of biopesticide spinosad against the cowpea weevil, *Callosobruchus maculatus* (F.) in west Africa. In the laboratory, Spinosad caused high mortality of adult *C. maculatus* and decreased the number of eggs laid by females. Spinosad, however, was less toxic in the 24 h treatment to *C. maculatus* than deltamethrin, an insecticide commonly used in Burkina Faso to control this insect. In "on-farm" experiments, Spinosad was effective in controlling *C. maculatus*. After 6 months of storage, the number of insects emerging from cowpeas seeds was reduced by >80% by coating seeds with Spinosad but only by 43% by coating with deltamethrin. Less than 20% of the seeds were perforated in the Spinosad treatment compared with 29% for deltamethrin. Spinosad controlled *C. maculatus* throughout the 6 months of cowpea storage whereas deltamethrin failed to control *C. maculatus* after 3 months of storage. Spinosad has the potential to be more effective in controlling *C. maculatus* than deltamethrin (Sanon *et al.*,2010).

Pathania and Thakur (2012) conducted laboratory studies against pulse beetle in stored black gram by using insecticides, viz. cypermethrin, deltamethrin, dichlorvos, fenvalerate and malathion. Malathion (0.5%), deltamethrin (0.1%) and cypermethrin (0.0025%) were found effective resulting in 100 per cent adult mortality after 7 days of exposure . Malathion (0.5%) was also proved to be the best in minimizing the oviposition (1.33 eggs/100 grains), seed damage (1.07%) and weight loss (0.28%) whereas fenvalerate (0.0075%) was least effective.

Rajjsri *et al.* (2012) evaluated the efficacy of neem products like neem oil and commercially available neem formulations viz., Econeem plus®, Neemindia ® and Neemazal ® in the laboratory for the control of pulse beetle, *C. chinensis* in stored bengalgram along with deltamethrin treatment as a chemical check. The bengalgram seed was treated with the neem formulations and stored under ambient conditions for storability studies. Observations on oviposition, insect damage, germination and seedling vigor index were recorded at three months interval. All the neem formulations were found to be effective against *C. chinensis* in stored bengalgram up to 12 months of storage. Deltamethrin treatment recorded highest oviposition and seed damage, resulted in poor germination and vigour of stored bengalgram seed indicating development of resistance in *C. chinensis* against commonly used synthetic pyrethroid insecticide deltamethrin in bengalgram. Crude neem oil @ 5 ml/ kg seed affected badly the germinability and seedling vigour of bengalgram seed. The neem formulations viz., Neemazal, Econeem plus and Neemindia were found to be very effective against *C. chinensis* in stored bengalgram and also maintained high viability and vigour of seed up to twelve months of storage. Our results suggest that these neem formulations can be used as safer alternatives to chemicals for long term storage of pulses.

Rahman *et al.* (2013) investigated the efficacy of botanical extract and chemical insecticide on the germination of pulse beetle *(Callosobruchus chinensis*) infested seeds of black gram (*Phaseolus mungo*), gram (*Cicer arietinum*), and mung bean (*Vigna radiata*). Neem extract (10%) and garlic extract (10%) were used as botanical extract where as 1% sevin 85 SP was used as chemical insecticide. It was observed that neem extract treated pulse seeds were germinated into highest number of normal seedlings in comparison with garlic extract and sevin 85 SP treated seeds when one day and ten days infested seeds were taken under consideration. But there was no significant difference in producing normal seedlings among neem extract, garlic extract and sevin 85 SP in case of thirtydays infested pulse seeds due to the fact that adult insect emerged and left the pulse seeds by this time.

2.2 Effect of botanicals on *Callosobruchus chinensis* and its impact on quality parameters in treated pulse seeds

2.2.1 Plant part powders/admixtures

Singh and Pandey (1995) reported that the seeds of black gram could be effectively protected from damage by *Callosobruchus chinensis* by mixing the seed with dried powder of neem cake at the rate of 100-400 mg/50g seed.

Chiranjeevi and sudhakar (1996) reported the effect of indigenous plant products on the fecundity, adult emergence and development of pulse beetle, in blackgram. Powder from neem (*Azadirachta indica*), sweet flag (*Acorus calamus*), apamarga (*Achyranthus aspera*), kesarachettu (*Crinum defixum*) and lantana (*Lantana camera*) and cow dung ash, *Acacia arabica*, *A. nilotica* wood ,neem wood and *Casuarina indica* wood ashes were tested for their effect on egg laying, development period and adult emergence of *Callosobruchus chinensis* infesting *Vigna mungo*.The application of 0.5-2.0 (w/w) neem seed powder completely prevented the development of the pest. Cow dung ash completely prevented the development at higher concentration.

Dhakshinamoorthy and Selvanarayan (2000) evaluated certain natural products against pulse beetle infesting stored green gram. The treatments comprised leaves as dried powder of various plants (neem, *Vitex negundo*, *Pongamia pinnata*, citrus and tulasi, fly ash, kitchen ash, castor oil, red earth, malathion (as standard control) and untreated control. Treated seeds were kept in plastic containers and 20 adult beetles were introduced into each container and kept covered with muslin cloth. The results revealed that the mortality of the beetle at 7 days after treatment was highest (100%) in castor oil, followed by neem leaf powder (91.66%).

Puneet *et al.* (2000) evaluated some edible plant products against the pulse beetle. Nine edible plant products viz anola (*Phyllanthus emblica*),black pepper (*Piper nigrum*), bitter gourd (*Momordica charantia*), clove (Synzygium *aromaticum*). Cinnamon, fenugreek, ginger red chili and turmeric were evaluated for toxicity against *Collosobruchus analis* infesting green gram (Vigna *radiata*). Maximum adult mortality after 7days exposure was recorded in clove powder treatment which also showed the minimum adult emergence and minimum number of eggs/100 grains after 5 months storage period.

Singh (2003) stated that seeds of Khesari could be effectively protected from the pulse beetle, *Callosobruchus chinensis* (L.) by mixing dried neem leaf powder @ 50 mg to 200 mg/100 g of seed. It provides good result in respect of toxic effect, safety and economy.

Yadav and Bhargava (2005) tested the four plant extracts viz., neem seed extract (*Azadirachta indica A. juss*) , undi extract (*Callophyllum inophyllum L.*) , karanj extract (*pongamia glabra Vent*), mustard oil (*Brassica juncea L.*) against *Callosobruchus maculatus* (Fab.). All the plant extracts caused significant mortality in adults after three days of treatment. The neem seed extract @ 1.0 ml/100g seeds were found to be most effective causing 49.175 mortality of adults. The mean mortality varied from 37.47 to 49.17% in different oils at the highest dose level of 1.0 ml/100g seeds. Neem seed extract @ 1.0 ml/100 g seed was the most effective in inhibiting the oviposition (21.67 eggs/ female) as against 82.67 eggs/ female in untreated seeds. At 1.0 ml/100g seeds, karanj extract caused maximum reduction in egg viability followed by neem seed extract (64.58%), undi extract (51.72%) and mustard oil (48.52%) . The longevity of both the sexes decreased with the increase in doses of plant extracts. No adverse effect of plant extracts was observed on the germination of moth bean seeds up to 90 days of treatment.

Govindan *et al.* (2009) studied the effect of plant powders on *Callosobruchus maculatus* (Fab). Among the plant powders evaluated , cent per cent mortality was observed in *Piper nigrum* @ 2 per cent (12 hr), *Anamirta cocculus* @ 2 per cent (96 hr) and *Paspalum Scrobiculatum* @ 2 percent (144hr) treated in black gram seeds. Egg laying was completely inhibited by *P.nigrum* (0.00) when compared to 137.00 eggs in untreated control. Adult emergence and seed weight loss was minimum (0.00%) in *P. nigrum* treated black gram seeds when compared to untreated control in which 38.22 per cent seed weight loss was observed.

Verma *et al.* (2009) evaluated five plant powders , viz. turmeric, ginger, black pepper, red chilli and aonla for the protection of French bean seed from pulse beetle, *Callosobruchus maculatus* (F.). One day after treatment, maximum mortality (40.00%) of *C. maculatus* was recorded in seed treated with black pepper at 5 per cent concentration. Black pepper powder resulted in maximum mortality which was 78.89 and 100 per cent after three and seven days of treatment, respectively. This was followed by turmeric (84.44%), red chilli(61.11%), ginger(54.44%) and aonla (50.00%) after seven days of treatment. The minimum number of eggs (20.39 eggs/ 100 seeds) was recorded with black pepper at 5 per cent concentration. Sixty days after treatment, minimum adult emergence (6.22 adults/100g seeds) observed in black pepper coated seeds was significantly different from other treatments. After 90 days of treatment, minimum adult emergence(12.78 adults/100g seed) was observed in black pepper followed by the turmeric (16.11 adults/100g seed) and the two were on par with each other.

Devi and Kalita (2011) evaluated the efficacy of some plant powders viz, tendril climber (*Melothria perpussilla*), black pepper (*Piper nigrum*), lemon grass (*cymbopogon flexuosus*), goat weed (*Ageratum Conyzoides*), china berry (*Melia Azedarach*), nirgundi (*Vitex negundo*), sweet basil (*Ocimum basilicum*), lantana (*lantana camara*), sweet flag (*Acarous calamus*) and Indian worm wood (*Artemisia nilagirica*) @ 3g/kg, 5g/kg and 10g/kg of green gram as post harvest grain protectants against pulse beetle, *Callosobruchus chinensis*. Based on the parameters like fecundity, number of adults emerged, percentage of grain damage and percentage of weight loss, sweet flag was observed the most effective closely followed by black pepper while goat weed , lemon grass and tendril climber were least effective.

Gill and Singh (2012) studied the efficacy of some plant powders against *Callosobruchus chinensis* on chickpea . Different plant powders , viz. leaf powders of neem, dharek, sadabahar and turmeric rhizome powder @ 2.0, 3.0 and 4.0 per cent were evaluated against pulse beetle, *C. chinensis* along with their effect on the germination of chickpea seeds. The results showed that the population build-up, per cent grain damage and per cent weight loss was significantly low in chickpea grains treated with turmeric rhizome powder @ 2.0, 3.0 and 4.0 per cent as compared to other treatments and untreated control when recorded at monthly interval upto four months of storage. However, all the plant powders at all the concentrations had no adverse effect on the germination of chickpea seedlings even after four months of storage.

Singh *et al.* (2012) conducted experiments against *Callosobruchus chinensis (L.)* on green gram seeds by using ten different plant powders viz., leaf and seed coat of *Zanthoxylum oxyphyllum* Edgw . ; seed of *Litsea citrate* BI.; seed coat and kernel of *Azadirachta indica A.Juss.;* leaf, seed coat and kernel of *Melia azedarach (*L.) and seed of *piper nigrum* L. @ 5% w/w and eight vegetable oils viz., neem , castor, clove, mustard, sunflower, sesamum, coconut, and groundnut oil @ 1% v/w as grain protectants. All the vegetable oils and *A indica* kernel powder provided maximum protection up to 2 months resulting in 2.22 to 9.11% seed infestation and 1.13 to 5.10% weight loss only as against 56.67 to 61.33% and 30.77 to 32.60% in untreated seeds. *P nigrum* and *L citrate* were fairly effective in reducing infestation and weight loss by about 60% as compared to untreated seeds up to 2 months , while *Z. oxyphyllum* was the least effective followed by *M. azedarach.* None of the plant materials adversely affected the seed germinability.

The study was taken up to assess locally available plant material viz. *Melia azadarach, Parthenium hysterophorus, Justica adhatoda , Vitex trifolia. Zanthoxylum acathopodium* and *Azadirachata indica* @ 5 g/40 seed of gram as grain protectants against pulse beetle, *Callosobruchus chinensis.* Among these *Azadirachta indica* and *Zanthoxylum acathopodium* were found more effective with 85.71% and 85.70% adults mortality after 7 days of treatment. The minimum number of eggs was recorded at 5g doses. At seventy days after treatment, minimum adult emergence (10.66 adults/40 seeds) was observed in *Azadirachata indica* leaf powder which was significantly different from other treatment followed by *Vitex trifolia, Justica adhatoda, Melia azadarach* and *P. hysterophorus*. The overall results indicated that neem leaf powder was found as effective grain protectants in suppressing the pulse beetle damage on gram (Bhubaneswari Devi and Victoria Devi , 2013).

Neog and Singh (2013) evaluated the plant powders viz., rhizome of *costus speciosus*, leaf of *Diospyros lanceafolia* Roxb., seed coat of *Diospyros lanceafolia*, leaf of *Juglans regia*, seed of *Litsea citrata*, leaf of *Ocimum sanctum*, seed of *piper nigrum*, leaf of *Zanthoxylum oxyphyllum* edgw.and seed coat of *Zanthoxylum oxyphyllum* @ 5% w/w as grain protectants against *Callosobruchus chinensis* (L.) on rice bean seeds. Based on the parameters like ovipostion, adult emergence, seed infestation and weight loss, *L. citrata* was observed the most effective up to 2 months of storage. It was closely followed by *P. nigrum*, *D.lanceafolia* and *J. regia* , while Z. *oxyphyllum* and *C. speciosus* were the least effective. These powders had no adverse effect on seed germinability. *L.citrata* and *P.nigrum* could reduce egg laying and adult emergence by 63.14 to 66.16% and 50.49 to 50.50%, respectively.

Patro *et al.* (2014) conducted laboratory experiment to test the efficacy of some spices against *Callobruchus chinensis* L. on stored gram seeds with six treatments including an untreated control . The data were recorded on days to cent per cent mortality, days to adult emergence, number of adults emerged and percentage damage to gram seeds. Cloves recorded minimum number of days to 100 per cent mortality (2.99), while maximum days (8.43) was observed in untreated control. The number of days to adult emergence ranged from 18.93 to 19.13 days in different treatments, minimum number (27.33) of adults being in cloves as against a maximum of 89.67 in control. Lower seed damage (3.67%) was also recorded in cloves, while the highest seed damage (13.67%) was calculated in control treatments.

Wast *et al.* (2015) investigated the efficacy of six plant materials namely bhilawa (*Semecarpus anacardium* L.), black gram flour (*Vigna mungo* L.), custard apple seed powder (*Anona squamosa* L.), neem leaf powder (*Azadirachta indica* A. Juss.), neem seed kernel powder and tobacco leaf powder (*Nicotiana tobaccum*) at two doses 0.5 mg and 0.25 mg per 100 g seeds (w/w) against *Callosobruchus maculatus* (Fab.) in the laboratory. The result revealed that the effectiveness of grain damage by *C. maculatus* was in the order of custard apple > neem seed kernel >

tobacco leaf > neem leaf > black gram flour > bhilawa. All the plant materials were found to be more effective at higher dose in comparison to the lower one. Further, all the plant material caused more than 17% egg mortality. The adult mortality ranged from 30.8% (tobacco leaf, 0.25 mg) to 50% (neem seed kernel, 0.5 mg). However, more than 30% reduction in seed weight has been observed in all the treatments.

2.2.2 Botanical oils

Yadav (1985) found that the neem seed oil @ 50 mg/ 10g seed *(Vigna radiata)* prevented oviposition by *C. maculatus* as compared with 40 mg/10g for *C.chinensis* and *C. analis.* Treatment at 30,10 and 20 mg/10 g suppressed all adult emergence of the three species respectively mainly due to toxic action against the eggs.

Ahmed *et al.* (1988) studied that the seeds of 6 varieties of green gram (*Vigna radiata*) were treated with mustard and olive oils to ascertain their efficacy as protectants against the bruchid *C. maculatus* and the impact of mustard oil on the germination of treated seed was also assessed. Ten grams of seed of each entry were placed in separate petridishes and treated with a measured quantity of oil to maintain dose of 15 ml of each oil/kg seed. Varieties treated with mustard oil showed complete protection to only 0.75% seed damage after 4 months.

Begum and Quiniones (1990) carried out experiments to test the efficiency of coconut, soyabean, mustard and groundnut oil in protecting stored seeds of *Vigna radiata* against the bruchid *Callosobruchs chinensis.* At dosages of 3 ml/kg, the oils were effective in protecting *V.radiata* against bruchid infestation and affected oviposition. Except mustard, none of the oils affected adult mortality. Oils at 3 ml/kg had a long residual effect reducing oviposition and inducing egg mortality even after 4 months.

Jacob and Sheila (1990) studied the effectiveness of crude palm oil, refined palm oil, sesame oil, neem oil and coconut oil against *Callosobruchus chinensis* in green gram. All the oils caused >60% mortality of the bruchid after 3 days , sesame and neem oil at 1 ml/100 g being the most effective.

Singal and Singh (1990) studied the efficacy of groundnut , coconut, mustard, sesame, soyabean and rapeseed oils as surface protectants at 1,3,and 5 ml/kg of seed against *Callosobruchus chinensis* on a variety of chickpea (*Cicer arietinum*) which resulted in significantly less oviposition on treated than on untreated seeds. Minimum percentage adult emergence (0.5) occurred from mustard oil treated seeds at 5 and 8 ml/kg followed by seeds treated with groundnut(7.6), soyabean (16.7) and rapeseed(6.2) oils at 1ml/kg. All the test oils adversely affected hatching and development of embryos, resulting in further population suppression.

Khaire *et al.* (1992) studied the efficacy of sunflower, castor, mustard safflower, palm, groundnut, sesame, neem, karanj and maize oil each applied at 5,7.5 and 10 ml/kg to protect pigeon peas from attack by *C. chinensis*. Effects on progeny emergence, loss in grain weight and seed germination up to 100 days after treatment were investigated. Adult emergence was prevented by karanj oil at 0.75 and 1.0% and neem oil at all concentration for up to 100 days.

The effect of 10 vegetable oils (sunflower,castor,mustard,safflower, groundnut,palm, sesame, neem, karanj and corn on ovipostional preference and egg hatching of *Callosobruchus chinensis* was tested on pigeon peas. All the treatments had an adverse effect on ovipositional preference compared with the control. Neem oil at all concentrations was superior to all the other treatments(Khaire *et al.*,1993).

Studies on the efficacy of 10 vegetable oils (sunflower, castor, mustard safflower, groundnut, palm, sesame, neem, karanj, and corn each at concentration of 0.5,0.75 and 1% as seed protectants of pigeon peas against *C. chinensis* were carried out to test the effect on oviposition and egg hatching at 33,66 and 100 days after treatment. The treatment with neem,castor,bean and karanj oils at 1% showed significant repellent action for egg laying by adult bruchids up to 100 days after treatment . No hatching of eggs at 33 days of storage was noticed in castor bean,neem,karanj and groundnut oils (Kachare *et al.*,1994).

Singh *et al.* (1994) reported that oils of sesame, sunflower, soyabean, linseed, mustard, safflower, karanj, castor, coconut,groundnut, ricebran and taramira were evaluated as grain protectants at 1 and 3 ml/kg seed of gram (*Cicer arietinum*) against *Callosobruchus chinensis*. In general the oil of taramira, coconut, sunflower, safflower and castor were found to be the most effective in inhibiting oviposition by the pest on gram seeds at both dosages as compared with the other oils. Seed treatment with oils usually delayed the development period of the insect by 6-14 days at both dosages. Adult emergence was lowest from the seeds treated with castor, mustard, soyabean, groundnut, coconut, safflower, taramira and ricebran oils at 1ml/kg seed, while there was no adult emergence from the seeds treated with oils of castor mustard, soyabean or taramira @3 ml/kg of seed.

Nine edible oils viz., sesame, cotton seed, mustard, rapeseed, groundnut, coconut, linseed, soyabean and safflower were evaluated @ 1,2 and 4 ml/kg as grain protectants against *Callosobruchus analis* infesting blackgram seeds. Cotton seed oil was the most effective followed by sesame, groundnut and coconut oil which resulted in low fecundity and prevented adult emergence for up to 150 days (Lakhanpal *et al.*, 1995).

Reddy *et al.* (1999) studied and reported that application of four plant oils viz., neem oil (*Azadirachta indica*), karanj oil (*Pongamia glabra*), mahua oil (*Maduca latifolia*) and Palmolein oil (*Elaeis guineensis*) at dosages of 0.5 and 1.0% level effectively protected green gram from pulse beetle,*C.chinensis*. These oils caused a significant reduction in oviposition and adults emergence. Neem oil at one percent level gave the best protection followed by palmolein, karanj and mahua oils.

Black gram seeds were treated with 3% dried leaf powders of *Vitex negundo, Aegle marmelos, Azadirachta indica, Datura stramonium, Ocimum sanctum, Lantana camera, Annona squamosal, Citrus limon* and fruit powder of *Capsicum annuum* and rhizome powder of *Acorus calamus* for control of *Callosobruchus chinensis* during storage. All treatments resulted in lower fecundity and seed weight losses than the untreated control. However, only the cow dung ash and mustard oil treatments completely inhibited oviposition and the *Vitex negundo, Aegle marmelos, Azadirachta indica* and *Datura stramonium* treatments reduced fecundity (Mishra,2000).

Six vegetable oils namely groundnut, sesame, soybean, coconut, mustard and neem were tested for their efficacy as repellant, ovipositional deterrent and ovicidal effect against pulse beetle, *Callosobruchus maculatus* (Fab.) in cowpea at one, seven, fifteen and thirty days after seed treatment (10 ml /Kg seed). Neem oil was found most effective and showed significantly higher repellent, ovipositional deterrent and ovicidal effect against the beetles followed by coconut, soybean and mustard oil, whereas groundnut oil was least effective. All the vegetable oils except neem lost their efficacy at thirty days after treatment (Bhatnagar *et al.*, 2001).

Laboratory study was undertaken to protect the pea grain from the attack of pulse beetle by mixing with indigenous plant products viz., leaf powder of lantana, sadabahar, neem, madar and kali tulsi @ 10 g/kg of grain and oil of castor, neem and mahua @ 2ml/kg grain to develop the safer products for its control. The result revealed that neem oil and neem leaf powder appeared to be most effective to minimize the damage by the pest in grains being 2.06 and 2.66 per cent, respectively, as against 69.63 per cent in untreated grains followed by the treatment of castor oil (2.87%). The loss in weight was as high as 47.40 % in untreated grains which considerably reduced to a level of 0.63, 1.00 and 1.10 % by application of neem oil, neem leaf powder and castor oil, respectively (Singh *et al.*, 2001).

Umrao and Verma (2002) assessed the efficacy of various plant products viz., leaf powder of Dharek and Sadabahar @ 10 g/kg grain and oils of coconut, mustard, groundnut and Neem products like Achook, Nimbicidine and Neem gold @ 1 ml/kg against pulse beetle, *Callosobruchus chinensis* on the basis of per cent grains damaged and per cent loss in weight. The Nimbicidine and Achook appeared to be most effective to minimize the damage by the pest in grains being 1.97 and 2.36 per cent, respectively as against 70.50 per cent in control followed by the treatment of neem gold (2.61 per cent). The loss in weight was as high as 45.20 per cent in untreated grains which considerably reduced to a level of 0.52, 0.93 and 1.07 per cent by application of Nimbicidne, Aschook and Neemgold, respectively.

Singh (2003) studied the effect of edible oils viz. coconut, mustard, sunflower, sesamum and mahua (*Madhuka indica L.*), non edible oil neem, karanj, castor, tarpin, and noorani as well as hair oil of arnica, himtaj, amla, banphool, and navratna as surface protectants for pegion pea seed against *Callosobruchus chinensis* (L.). These oils @ 8 ml/kg of seed proved highly effective in protecting the seed up to 9 months storage in term of seed damage and weight loss. These oil prevented egg laying and checked population build up of beetle.

Singh and Yadav (2003) evaluated the efficacy of six different oils viz., neem, mehendi, castor, karanlj, mustard, and olive against *Callosobruchus chinensis* in green gram in a laboratory experiment. The seed coating of these oils gave significant protection against *Callosobruchus chinensis* as compared to untreated control. The oil coating 6-8 hrs. after treatment at doses of 2.5,5 and 10 ml/kg seeds gave partial to complete protection at 90, 150 and 210 days after treatment.Neem and mehendi oils at the dose of 10 ml/kg seed were effective even beyond 150 days after treatment and rest of the oils were effective even after 280 days of oil treatment . The germination test was also carried out with the oil treated seeds (6 to 8 h, 90, 150 and 210 days after treatment) where , the treated seeds were given 8h. exposure to sun showed non-significant differences among treatments as well as in untreated control.

Efficacy of various grain protectants, viz. neem seed kernel powder @ 20g/kg, neem oil@10ml/kg , mustard oil@ 7.5ml/kg, groundnut oil @7.5ml/kg, turmeric powder @3.5 g/kg, mustard oil + turmeric power @3.75 ml 1.75 g/kg, groundnut oil +turmeric powder@ 3.75 ml + 1.75g/kg, 7 cm covering of saw dust, sand, dung cake ash and wheat husk in each was evaluated on adult emeregence holes and seed damage by pulse beetle, *Callosobruchus chinensis* on pigeonpea, *Cajanus cajan* at 45, 90 and 135 days after storage. No adult emergence holes were observed and per cent seed damage was nil in treatments of 7 cm covering of saw dust , sand, dung cake ash and neem oil@10 ml/kg seeds at all the storage intervals (Jangamashetti *et al.*,2008).

Sreekanth *et al.* (2011) investigated the efficacy of different plant products and vegetable oils against the bruchid, *C. chinensis* (*L.*) in Rajma beans in laboratory and found that coconut oil followed by niger and sesame oil at the concentration of 1.5 ml/100 g seed proved their superiority over all other plant products in inducing higher mortality at three days and 180 days after treatment. The bruchids failed to lay eggs and there by no adult emergence and no weight loss in coconut oil treated seeds were recorded even after 180 days of treatment. Germination of the seeds was not affected by any of the treatments at three days and 180 days after treatment. Among all the treatments, oils proved to be better. Hence it is concluded that oils especially coconut oil followed by niger and sesame oil as per availability may be recommended for checking *C. chinensis* in Rajma beans.

2.2.3 Botanical formulations

Banjo and Mabogunje (1999) studied the relative effectiveness of extracts of *Azadirachta indica* (leaf) physics plant , *Jatropha curcas* (leaf and seed), *Piper nigrum* (fruit) and *Ocimum canum* (leaf and seed) for the control of bean weevil (*Callosobruchus maculatus*) in stored cowpea. Extracts from *J.curcas* seed was the most effective against the pest followed by *J. curcas* leaf and *P.nigrum* fruit. Other plant extracts were ineffective.

Kiradoo *et al.* (2004) evaluated the efficacy of *Ocimum sanctum* and *Ocimum basilicum* (Lamiaceae) on egg-laying performance of the bruchid on grains of *Vigna radiata* (mung). The leaves of the selected two plants were employed in the form of crude extract, powder suspension, aqueous extract, ethanol extract and diethyl ether extract at dose concentrations of 10, 25 and 50%. Significant reduction (P< 0.05) in oviposition was documented in sets treated with the formulations of both the plants as compared to control and normal. The formulations of *O. basilicum* were observed to be superior to *O. sanctum* in reducing egg-laying. Moreover, ethanol extract, crude extract and powder suspension were also noted to significantly bring down the number of eggs laid by the pulse beetle. Dose concentration was found to be inversely proportional to the number of eggs laid by the bruchid and formulations of 50% concentration resulted in minimum oviposition. The results suggested that formulations of both the species of *Ocimum* have a potential to act as ovipositional deterrent and can be employed against *C.chinensis* for its control.

Efficacy of plant extracts as grain protectants (methyl extract of *Erigeron karwinskyanus, E. liniforms, E. canadesence, Blumea mollis, Myriactis javonica and Bidens pilosa*) was evaluated on mung bean @ 1.0% as an alternative source of synthetic pesticides. Among these *Blumea mollis* was found to be most effective for controlling oviposition and adult emergence of pulse bettle (Tandon *et al.*, 2004).

Mandal *et al.* (2005) investigated the efficacy of malathion and various indigenous plant products mixed in water, ethanol, methanol and acetone against fecundity of pulse beetle, *Callososbruchus chinensis L.* in stored seeds of bengal gram, *Cicer arietinum L.*The insecticide malathion 50 EC@ 1ml/litre of water only as surface treatment was found to be the most effective where no fecundity of this beetle was recorded. Among the plant extracts neem (*Azadirachta indica*) in water, ethanol, methanol and acetone was observed to be the most effective in terms of lowest fecundity of bruchid in stored seeds of bengal gram followed by nisinda (*Vitex negundo*) and ipomea (*Ipomea carnea*) , respectively in 1, 3 and 6 hours after treatment. Leaf extract of pudina was also recorded to be effective to some extent in decreasing the egg laying of this beetle.

Mishra *et al.* (2006) the solvent extracted vegetable seed oils of Cucurbitaceae family viz. bitter gourd (*Momordica charentia*), small bitter gourd (*Momordica* *dioica*), bottle gourd (*Lagenaria siscraria*) and ridge gourd (*Luffa acutangula*) against *callosobruchus chinensis* on the stored legume-pulse grains. All the vegetable seed oils were found effective as grain protectant, which provided negligible weight loss at the oil-application rate of 6-8 ml/kg in legume-pulse grain after 60 days storage at laboratory conditions.

Rahman *et al.* (2006) studied the bio-efficacies of different plant/weed derivatives against the pulse beetle, *Callosobruchus maculatus* F. on black gram seeds. Plant extracts, powder, ash and oil from nishinda (*Vitex negundo* L.), eucalyptus (*Eucalyptus globules* Labill.), bankalmi (*Ipomoea sepiaria* K.), neem (*Azadirachta indica* L.), safflower (*Carthamus tinctorius* L.), sesame (*Sesamum indicum* L.) and bablah (*Acacia arabica* L.) were evaluated for their oviposition inhibition, surface protectant, residual toxicity and direct toxicity effects on *C. maculatus*. The least number of F_1 adults emerged from black gram seeds treated with neem oil. The nishinda oil extract was the most toxic of three extracts tested (nishinda, eucalyptus and bankalmi). Bablah ash was the most effective as compared to the powdered leaves of nishinda, eucalyptus and bankalmi. The powdered leaves and extracts of nishinda, eucalyptus and bankalmi at a 3% mixture, provided good protection for black gram seeds by reducing insect oviposition, F_1 adult emergence, and grain infestation rates. The oil treatment did not show adverse effects on germination capability of seeds, even after three months of treatment.

Braga *et al.* (2007) studied the the effect of 2-tridecanone vapor on the cowpea weevil,*Callosobruchus maculatus*. Seeds of cowpea were infested with adults and exposed to different doses of 2-tridecanone isolated from *Pilocarpus microphyllus* Stapf ex Holm, a plant species native to north-eastern Brazil. The pure monoterpene was evaluated both undiluted as well as in the dilutions 1:10, 1:100 and 1:1,000 (v/v).The vapor of 2-tridecanone caused a significant ($P < 0.05$) reduction in the number of eggs laid, in the percentage of eggs hatched and in the number of emerged adults in infested seeds. The fumigant insecticidal effect of 2-tridecanone was mainly due to its ovicidal activity.

Govindan and Nelson (2007) conducted laboratory study on the effect of *Acorous calamus* rhizome powder and its dust formulations on *Callosobruchus maculatus*. Cent per cent mortality of *C. maculatus* was recorded in black gram treated with *A. calamus* rhizome powder @ 0.75, 1.00, 1.25 and 2.00 per cent at 48 hr after the treatment. *A. calamus* dust formulations were made using talc and fly ash as filler, *A. calamus* 10D (talc as filler) (Ac 10 DT) caused 100 per cent mortality at 3.50 per cent after 72 hr of the treatment. *A.calamus* 10D (fly ash as filler) (Ac 10 DF) caused 100 per cent mortality after 84hr of the treatment.

Chaubay *et al.* (2008) determined the insecticidal, oviposition, egg hatching and developmental inhibitory activities with the essential oil from seven common spices, *Anethum graveolens, Cuminum cyminum, Illicium verum, Myristica fragrans, Nigella sativa, Piper nigrum* and *Trachyspermum ammi* against pulse beetle, *Callosobruchus chinensis.* Essential oils were isolated by hydro-distillation method using Clevenger apparatus. These essential oils caused death of adults and larvae of *Callosobruchus chinensis* when fumigated.These essential oils reduced the oviposition potential, egg hatching rate, pupal formation and emergence of adults of F(1) progeny of the insect when fumigated with sublethal concentrations. These essential oils also caused chronic toxicity as the fumigated insects caused less damage to the stored grains. The essential oil of N. sativa was found most effective against all the different stages of the *Callosobruchus chinensis* followed by *A. graveolens, C. cyminum, I. verum, P. nigrum, M. fragrans and T. ammi* oils. All the responses were found concentration-dependent. The toxic and developmental inhibitory effects may be due to suffocation and inhibition of various biosynthetic processes of the insects at different developmental stages.

Different solvent extracts (hexane, dichloromethane, ethyl acetate and water) of eight plants were evaluated for their insecticidal and oviposition deterrent activity against *Callosobruchus maculatus.* Hexane extract of *Swientenia mahagoni* (8.8+3.96) and *Pedalium murex* (21.4+12.34), dichlorometane extract of *Diospyros ovalifolia* (12.8+4.65) and *P. murex*(12.2+8.16), ethyl acetate extract of *Prosopis juliflora* (8.6+4.15) and *Calophyllum inophyllum*(8.8+7.59), water extract of *Artemisia pallens*(10.4+6.12) and *D. ovalifolia* (14.8+8.22) exhibited the maximum oviposition deterrent activity. Insecticidal activity was maximum in hexane extract of *p. murex* (65.6%), dichloromethane extract of *P. juliflora* (58.4%), ethyl acetate extract of *S. mahagoni* (59.2%) and water extract of *P. murex* (44.6%) and other plant extracts tested showed 3.0 to 41.6% of mortality (Raja *et al.*, 2009).

Balachandra *et al.*(2011) conducted an experiment against bruchid, *Callosobruchus maculatus* (F.) causes major losses during the storage of cowpea seeds [*Vigna unguiculata* (L.)Walp.]. Essential oil isolated from *Plectranthus zeylanicus* plant was tested for potential insecticidal activity against *C. maculatus*. The gas chromatography studies of the essential oil of *P. zeylanicus* showed that ρ-cymene (3.5%), β-caryophyllene (0.2%), geranyl acetate (9.3%) and geraniol (7.2%) were the major constituents. The adults of *C. maculatus* were susceptible to both fumigant and contact toxicity of *P. zeylanicus* plant oil. LC(50) values of 0.927 and 0.010 g L(-1) were obtained for fumigant toxicity and contact toxicity assays, respectively. Oviposition and F(1) adult emergence were significantly inhibited by *P. zeylanicus* plant oil at a concentration higher than 0.001 g L(-1) in both fumigant and contact toxicities. The analysis of olfactometer and choice camber bioassays revealed the repellent effects of the oil of *P. zeylanicus* plant.

Chaubay *et al.* (2013) investigated the efficacy of essential oils of *Zingiber officinale* and *Piper cubeba* for repellent, insecticidal, anti-ovipositional, egg hatching, persistence of its insecticidal activities against pulse beetle, *Callosobruchus chinensis*. Essential oil vapours repelled bruchid adults significantly as oviposition was found reduced in choice oviposition assay. *Z. officinale* and *P. cubeba* essential oils caused both fumigant and contact toxicity in *C. chinensis* adults. In fumigation toxicity assay, median lethal concentrations (LC50) were 0.34 and 0.27 microL cm(-3) *for Z. officinale* and *P. cubeba* essential oils, respectively, while in contact toxicity assay, LC50 were 0.90 and 0.66 microL cm(-2) for *Z. officinale* and *P. cubeba* essential oils, respectively. These two essential oils reduced oviposition in *C. chinensis* adults when treated with sublethal concentrations by fumigation and contact method. Oviposition inhibition was more pronounced when adults come in contact than in vapours. Both essential oils significantly reduced egg hatching rate when fumigated. Persistence in insecticidal efficiency of both essential oils decreased with time. P. cubeba showed less persistence than *Z. officinale* essential oil because no mortality was observed in *C. chinensis* adults after 36 h of treatment with *P*. cubeba and after 48 h of treatment with *Z. officinale* essential oil. Fumigation with these essential oils had no adverse effect on the germination of the cowpea seeds. Findings of the study suggest that *Z. officinale and P.cubeba* essential oils can be useful as promising agent in insect pest management programme.

Singh and Jakhmola (2014) observed that among 45 treatments ; petroleum ether extract of margosa seed induced significantly highest cumulative mortality (53.35%) in *Callosobruchus maculatus*. Egg deposition by *C. maculatus* was increased in all the plant extracts with the increase after release up to 7 days. It indicated that effectiveness declined in treated seeds with passage of time. The maximum repellency was recorded in petroleum ether of margosa seed at all the intervals under seed treatment and filterpaper strip methods.

2.3 Qualitative and nutritional changes due to infestation by bruchids in pulse seeds

Umrao and Verma (2003) studied on protein composition of 20 pea genotypes for preference of *Callosobruchus chinensis* Linn. It was found that low protein content 21.51, 21.87, 22.06, 22.07, 22.09 and 22.47 of respective genotypes on DMR-29, KPMR- 186, HTJP-2, KPMR-347, KPMR-327, and HUDP-10 were least susceptible while, higher protein 24.24, 23.72, 23.61, 23.53, 23.42 and 23.24 per cent on genotypes HUP-13, MDP-3, MDP-1, KPMR-389, KPMR-157 , KPMR-397 respectively, highly susceptible to pulse beetle. After 120 days of infestation, the protein content of each pea variety significantly increased due to infestation of *C. chinensis*. The relationship of fresh grain protein with the infestation(fecundity,F_1 progeny and index susceptibility)was positive and significant. It means the increased protein contents of the pea genotypes certainly increased the infestation.

Singh *et al.* (2004) screened different varieties of pulses against *C chinensis* using growth index values as the parameters for susceptibility. In all pulses, the varieties with higher growth index values (i.e. higher susceptibility) have the lower protein content i.e. susceptibility to *C. chinensis* of a pulse variety is negatively correlated with its protein content. The only exception in the present study was lentil, where the protein content values of the most and least susceptible varieties are very close (25.56 and 25.1%) to draw any conclusion.

Correlation studies were carried out between physical characters of eleven different pulses viz.,green gram, black gram, lentil, chickpea (deshi), chickpea (kabuli), French bean, pea, pigeon pea, cowpea,soybean and rice bean and oviposition by *Callosobruchus chinensis* (L.) in multiple and restricted choice conditions. It was also studied to find out any possible relationship of both physical and chemical characters with the host suitability and preference of the pest. The seed characters such as 100 seed weight, seed coat thickness, colour and texture of seed coat were not related with the ovipositional preference and host suitability of the pest to different pulse seeds. However, the biochemical characters such as total soluble sugar, reducing sugar, non- reducing sugar and amylase fraction of starch had a positive influence on host suitability (in terms of growth Index) and preference(in terms of seed infestation) of *C. chinensis*. The starch, crude protein, free amino acid, crude oil and free fatty acid content were not significantly correlated with any of the developmental parameters of the pest (Neog and Singh,2011).

Neog and Singh (2011) carried out qualitative studies in the seeds of different pulses due to infestation by *Callosobruchus chinensis* (L.) after 60 and 90 days of storage revealed that the total soluble sugar, non reducing sugar, starch and crude fat content decreased; while the moisture, reducing sugar, crude protein, free fatty acid and uric acid content increased significantly in the infested seeds as compared to healthy ones. The corresponding increase or decrease of these biochemical contents was the highest in green gram and the lowest in soybean. The qualitative changes in the seeds of these pulses were directly related to the level of insect infestation.

Pal *et al.* (2011) studied differential reaction of *Callosobruchus chinensis* L. in green gram varieties. Varieties with low protein and higher oil content were least susceptible whereas those with high protein content and low oil content were highly susceptible. PUSA- 9871 (24.38%), TM-9937(22.50%), HUM- 12 (22.50%) had higher protein content , coupled with oil content 0.82, 0.88 and 0.92%,showed high susceptibility. Varieties with lower protein content and with higher oil content like PDM 96-261, PDM-199, SML-668 were moderately resistant. There was considerable reduction in protein content after *C. chinensis* feeding. The protein content of the varieties had highly significant positive relationship with fecundity, F1 progeny and index of susceptibility. Higher protein content in fresh seeds of the varieties led to more infestation while less oil content in healthy seeds varied from 0.82 to 1.43 % being minimum in PUSA-9871 and maximum in PDM-96-261. Considerable reduction in oil content in the varieties of green gram was noticed after the infestation of *C. chinensis.* The oil content of healthy seeds had highly significant negative correlation with fecundity, F1 progeny and index of susceptibility.

CHAPTER-III

MATERIALS AND METHODS

MATERIALS AND METHODS

Studies were conducted in the laboratory of Seed Technology Research Centre, OUAT, Bhubaneswar to evaluate the performance of some new molecules and botanicals against pulse beetle, *Callosobruchus chinensis* L. on stored green gram and black gram seeds. Different trials were carried out during 2014-15 by exposing the pest at different time intervals during storage on both the pulse seeds treated with various plant part powders, oils ,formulations and chemicals. During the course of investigation the residual toxicity of chemicals and botanicals against pulse beetle on percentage of mortality, number of eggs laid per 200 seeds, number of adults emerged, percentage of seed infestation and its subsequent effect on seed quality parameters were assessed. Protein content and percentage weight loss of treated green gram and black gram seeds exposed to pulse beetle infestation after 6 months of storage were also analysed. Materials and methods pertaining to present studies are mentioned below.

3.1 Materials

3.1.1 Test insect

Pulse beetle, *Callosobruchus chinensis* (L.) (Bruchidae: coleoptera).

Three species of pulse beetle i.e., *C. chinensis*, *C. maculatus* and *C. analis* infest pulses in Odisha. Among them *C. chinensis* (L.) is the most commonly encountered species on coastal climatic condition of Odisha inflicting serious damage to the stored seeds of legumes both quantitatively and qualitatively. It is a notorious storage pest and attacks wide range of pulses through out tropical and subtropical area. The main hosts are *Vigna radiata* Linn., *V. unguiculata* L. Walp., *Cajanus cajan* L. Millisp., *Cicer arietinum* Linn., *Vigna mungo* L. Hepper., *Pisum sativum* Linn., *Lathyrus sativus* Linn., *Dolichos biflorus* Roxb. Etc. Due to short life cycle and high degree of reproductive capacity losses caused by this beetle to pulses have been estimated to the tune of 40-50 % in storage (Shaya *et al.*, 1997). All three species of genus *Callosobruchus* have similar resemblance and difficult to differentiate in naked eye. Minute and critical observation is necessary to identify different species of pulse beetle (Fig.1).

3.1.2 Test seeds

Matured, well filled and healthy pest free green gram (*Vigna radiata*, Barchana local) and black gram (*Vigna mungo*, Barchana local) seeds were procured from farmers' of Barchana village for experimental purpose (Fig. 2 and 3).

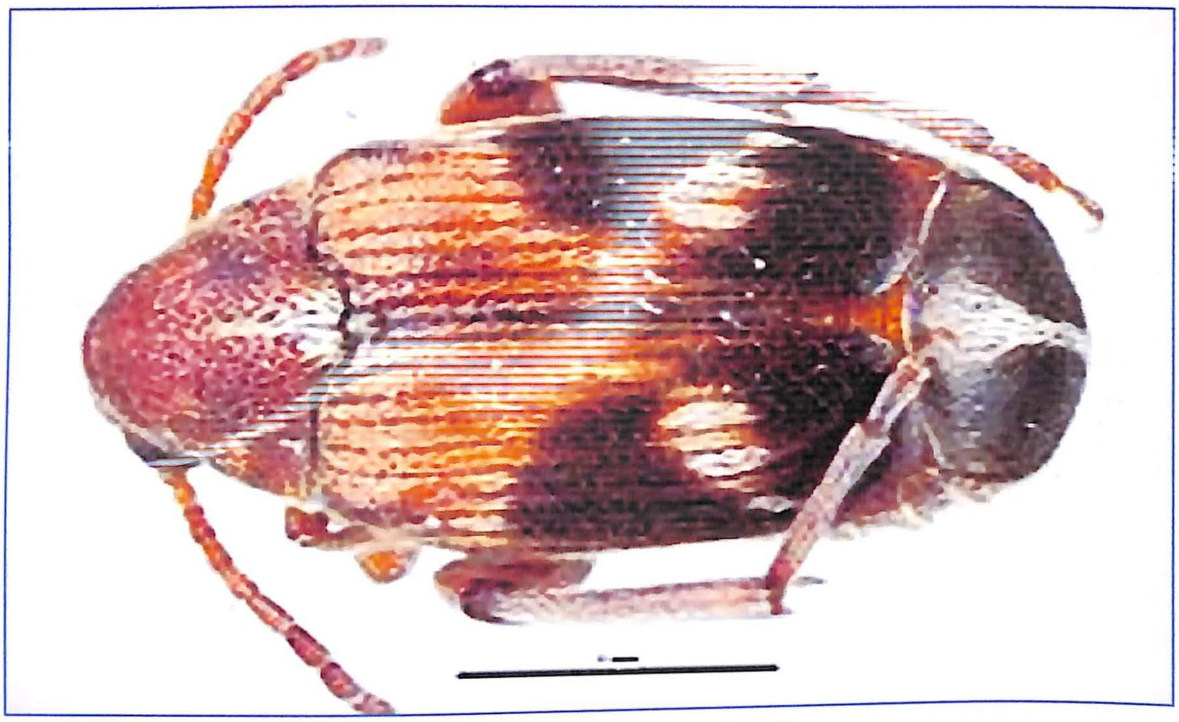


Fig. 1 Test insect, pulse beetle

Fig. 2 Test seed of green gram

Fig. 3 Test seed of black gram

3.1.3 Apparatus used

i) Rearing jar of size 20 x 10 cm for rearing of test insects

ii) Muslin cloth to cover the rearing jars

iii) Rubber band used to tie the muslin cloth tightly on the rearing jars and plastic jar to avoid the escape of adults.

iv) **Aspirator** for collection of small flying adult beetles. A rubber cork having 2 holes of size 1.5 mm diameter was tightly fitted to a wide mouthed conical flask. Two glass tubes of 18 and 20 cm length of 90° and 180° angle respectively inserted through those holes for convenient handling of insects.

v) Big round bottom flasks used for storing the seed materials to provide food while rearing the test insects.

vi) Plastic jars of size 10 x 7 cm were used for carrying out the mortality test.

vii) Physical balance and weight box were used to weigh pulse seeds, botanicals (plant materials) and insecticides (granular and semisolid form).

viii) Electrical oven with temperature limit of 200°C were used for determination of moisture content of seeds.

ix) Petridishes of 10 cm diameter were used for conducting germination test, counting number of damaged and undamaged seeds, number of eggs laid/200 seeds.

x) Graduated micropipetts of different capacity were used to measure required quantities of insecticides (ppm/µl) for seed treatment.

xi) Magnifying glass

xii) Measuring cylinder was used to take required quantity of water for dilution of insecticides as per quantity of seed material.

xiii) Hand globes for mixing of insecticidal solution thoroughly with seeds.

xiv) Other materials like needle, marker pen, forceps, scissors, ethanol, hair brush, cotton etc. were required during the period of investigation.

3.1.4 Test insecticides

The following insecticides were used for conducting experiment. Chemical name, trade name and manufacturing company of test insecticides are mentioned below (Table 1).

Table 1. Detailed information on test insecticides

Sl. No.	Chemical name	Trade name	Manufacturing company
1	Emamectin benzoate	Elpida 5 SG	Modern papers, Phase-1, SIDCO Industrial Complex, Jammu-181133, J&K
2.	Spinosad	Tracer 45 SC	DOW Agrosciences India Pvt. Ltd, 1st floor, Block-B, 0-2 Godrej Business Dist., PirojshaNagar, LBS Marg,Vikhroli, West Mumbai-400079
3.	Profenofos	Carina 50 EC	PI Industries Ltd, 237, GIDC Panoli, Ankleshwar-394116,Dist-Bharuch, Gujarat.
4.	Novaluron	Rimon 10 EC	Makhtheshim Chemicals Work Ltd, POB 60, Beer-Sheva-84100, Israel.
5.	Deltamethrin	Decis 2.8 EC	Punjab Pesticides Industrial Coop. Society Ltd, Village Khanpur, Kharar, Dist-Ropar, Punjab.

3.1.5 Test botanicals

The following botanicals were used for conducting experiment

Table 2. Detailed information on botanicals

Sl. No.	Common name	Scientific name	Family	Preparation used
1.	Begunia	*Vitex negundo*	Lamiaceae	Leaf powder
2.	Naguari	*Lantana camera*	Lamiaceae	Leaf powder
3.	Curry leaf (Bhusunga)	*Murrya koenigii*	Rutaceae	Leaf powder
4.	Citronella	*Citronella mucronata*	Cardiopteridaceae	Oil
5.	Sweet flag	*Acorus calamus*	Acoraceae	TNAU Formulation 6 EC

3.2 Methodology

3.2.1 Rearing of test insect

In order to provide steady supply to test insects (newly emerged adult beetles) at regular intervals throughout the period of investigation, rearing of pulse beetles was carried out at Seed Entomology laboratory of the Department of Seed Science and Technology,OUAT,Bhubaneswar. Prior to start the rearing of test insects, green gram and black gram seeds were first fumigated with aluminium phosphide to eradicate associated insects and mites if any present. The food stock was kept separately in big round bottom flasks in order to supply healthy seeds to the newly emerged adults beetles depending upon the intensity of damage of seeds in the rearing jar.

3.2.2 Rearing technique

Initial or foundation culture of pulse beetles, *Callosobruchus* spp. was obtained from infested mung bean seeds. The seeds containing eggs of pulse beetles were transferred to separate jar. The beetle started emerging from infested mungbean seeds after 20 days. Rearing of beetles was undertaken separately in plastic jars of 1 kg selected green gram and black gram seeds at appropriate 10.5 % moisture content. In each jar 50 pairs of freshly emerged adult beetles were released with the help of an aspirator and muslin cloth was tied tightly at top of jar with help of rubber band to prevent escape of insects. Rearing jars are kept undisturbed in dark place for 1 week during which the females laid eggs on seeds. After 7 days of oviposition period, the adults were removed and eggs were allowed to incubate in laboratory. A large number of adult beetles emerged within a period of 20 to 25 days. Similar procedure was followed to get new culture subsequently at regular intervals which were used at appropriate time of requirement during period of study. Care was always taken to have sharp eye on emergence of beetles on the rearing jar in order to ensure timely supply of test insects to be utilized for experimental purpose (Fig. 4).

Fig. 4 Mass culture of pulse beetle

3.2.3 Collection of test insect

Adult test insects, *C. chinensis* (L.) were collected by help of aspirator because it was difficult to collect by hand due to quick moving and flying nature of insects and there were chances of damage/breakage to body parts. The rearing jars with newly emerged adults were kept in an insect collection glass cage and muslin cloth tied over the mouth of jar was removed. The adult beetles came out of the jar and moved on glass walls of the collection cage. In this way desired number of insects were collected by aspirator and used for study.

3.2.4 Insecticidal management of pulse beetle seed

Seed : Green gram and black gram (Barchana local)

Table 3. **Chemicals as seed protectant**

Treatment	Chemicals	Concentration
T_1	Emamectin benzoate (Elpida 5 SG)	2 ppm (40.0 mg/kg seed)
T_2	Spinosad (Tracer 45 SC)	2 ppm (4.4 mg/kg seed)
T_3	Profenofos (Carina 50 EC)	2 ppm (0.004 ml/kg seed)
T_4	Novaluron (Rimon 10 EC)	5 ppm (0.05 ml/kg seed)
T_5	Deltamethrin (Decis 2.8 EC)	1 ppm (0.04 ml/kg seed)
T_6	Untreated control	-

Time of release : 1 day, 2, 4 and 6 months after seed treatement.

Quantity of seeds per vial (10 x 7 cm) : 50 g

Number of replications : 4

Experimental design : CRD

Experimental condition : Ambient

There were 6 treatments comprising 5 insectides and one untreated control. The laboratory experiment was designed in CRD and each treatment was replicated 4 times. The insecticides were procured from local market.

3.2.4.1 Disinfection of test seeds

Freshly harvested seeds of green gram and black gram procured from farmers were completely dried under the sun in order to reduce moisture content below 10 per cent. The seeds were then fumigated separately in enclosed chamber for 7 days by aluminium phosphide tab @ 1 tab (3 g/m^3 space) to destroy the insect pests. After fumigation the entire seed lot was aerated so as to remove any trace of phosphine gases. A total of 24 kg a.i., 12 kg each of green gram and black gram seeds were fumigated for conducting both insecticidal and botanical experiments.

3.2.4.2 Quantity of seeds requirement for each treatment (1 kg of green gram and black gram seeds each).

Laboratory experiments for insecticidal treatments were conducted separately for green gram and black gram seeds. Required quantity of insecticide (SG/SC/EC formulation) was diluted with 5 ml of water andmixed thoroughly with 1 kg seed after spreading on polythene sheet. Separate polythene sheets were used for different treatments. In control, only 5 ml of water was mixed with the seeds Then both green gram and black gram treated seeds were dried separately treatment wise under fan at room temperature under same polythene sheets used for mixing the insectides.

3.4.3 Filling of insecticidal treated seeds in plastic vials/jars.

The treated seeds were filled in 12 vials (20 x 15 cm), 6 vials for green gram and 6 vials for black gram seeds @ 1 kg/vial and kept as stock and stored. The cover of vials were tightly fitted in order to prevent natural infestation by bruchids. Treatment number (T_1, T_1 .... etc.) and seed name were clearly mentioned on jar in order to avoid confusion. From the stored stock seeds, 200 g of green gram and black gram seeds were removed treatment wise one day after insecticidal treatment. Each 200 g seeds were divided into 4 parts @ 50 g seeds which formed four replication of each treatment. Each 50 g of treated seeds were transferred to a plastic jar (10 x 7cm). Altogether there were 24 vials for green gram and same number of vials for black gram seeds for initial laboratory experiment. Similarly from stored stock of insecticidal treated seeds, 200 g of green gram and black gram seeds were removed treatment wise after 2, 4 and 6 months of seed treatments for conducting 2nd, 3rd and 4th phase of laboratory experimentss following same procedure mentioned above using the same 24 vials each for green gram and black gram seeds. The extra left over seeds were discarded during final phase of laboratory experiment i.e., 6 months after treatment.

3.2.4.4 Release of test insects

10 pairs of freshly emerged *C. chinensis* adults aspirated from rearing jar and were released in each vial filled with treated seeds stored for 24 hours, 2, 4, and 6 months after seed treatment for conducting initial, 1st, 2nd and 3rd phase of laboratory experiments, respectively. Then top of vials were tightly covered with muslin cloth by the help of rubber bands. The vials were kept undisturbed for 7 days.

3.2.5 Management of pulse beetle by botanicals

Table 4. Botanicals as seed protectant

Treatment	Botanical	Concentration
T_1	*Vitex negundo* (Begunia leaf powder)	10 g/kg seed
T_2	*Lantana camera* (Naguari leaf powder)	10 g/kg seed
T_3	*Murrya koenegii* (Curry leaf powder)	10 g/kg seed
T_4	*Citronella mucronata* (Citronella oil)	10 ml/kg seed
T_5	*Acorus calamus* (Sweet flag formulation)	10 ml/kg seed
T_6	Untreated control	-

Time of release : 1 day, 2, 4 and 6 months after treatment during storage

Quantity seeds/vial : 50 g

Number of replication : 4

Experimental design : CRD

Experimental condition : Ambient

There were 5 botanicals (leaf powder, oil/formulation) used for seed treatment as protectant against *C. chinensis* and 1 untreated control. Altogether laboratory experiment comprised 6 treatments replicated 4 times.

3.2.5.1 Disinfestation of test seed

Disinfestation of total quantity of green gram and black gram seeds (12 kg of green gram and black gram seeds each) required for conducting both insecticidal and botanical laboratory experiments treatment was done simultaneously prior to seed treatment as per procedure mentioned in 3.2.4.1.

3.2.5.2 Quantity of seeds required for each treatment (1 kg for green gram and black gram seeds each)

Botanicals i.e., begunia, naguari and curry leaves collected and dried under shade and ground to fine powder of 60 mesh sieve. Citronella oil was purchased from local market. Sweet flag formulation (6 EC) was prepared and supplied by TNAU. These botanicals (admixtures/oil/ formulation) were thoroughly mixed with both the pulse seeds separately @ 10 %/kg of seed. In control treatment only 5 ml water was mixed with per kg seeds. Mixing process was done by spreading a polythene sheet treatment wise for both pulse seeds separately. Green gram and black gram seeds mixed with citronella oil and sweet flag formulation were shade dried under fan at room temperature in Seed Entomology Laboratory.

3.2.5.3 Filling of botanicals treated green gram and black gram seeds in plastic vials.

Similar procedure followed as indicated in 3.2.4.3 section for insecticidal experiment.

3.2.5.4 Release of test insects

Similar method followed for release of test insects in botanicals treated both pulse seeds as mentioned in 3.2.4.4 section.

3.2.6 Experimental observations

Observations on per cent mortality of pulse beetles, number of eggs laid/200 seeds, number of adults (F_1 progeny) emerged and seed quality parameters like seed infestation (%), germination (%), moisture content (%), vigour index etc. were recorded at different storage periods/ time intervals in storage after seed treatment. Similarly weight loss and protein content of treated green gram and black gram seeds artificially infested with pulse beetle after 6 months storage period were also determined.

3.2.6.1 Residual toxicity

Observations on mortality (%) of adult beetles on treated seeds at different storage after seed treatment was taken to assess the residual toxicity of test insecticides and botanicals against pulse beetle. Observations on mortality (%) of adult beetles exposed to treated seeds at different time intervals after (1 day, 2, 4 and 6 months of storage period) were recorded after 7 days of release by noting the number of live and dead insects. The moribund insects were considered as dead. Then they were removed from the jar. Mortality was computed by using the formula mentioned below.

$$\text{Mortality (\%)} = \frac{\text{Number of dead insects}}{\text{Total number of insects released}} \times 100$$

3.2.6.2 Oviposition

Number of eggs laid was observed by taking 200 randomly selected seeds of both pulses from each treatment replication wise and counting the number of eggs at 7 days after release of adult beetles. Small white dot like appearance was observed on oviposited seeds (Figure 5).

Fig. 5 Eggs of pulse beetle

3.2.6.3 Population build-up of adult beetle

Observation on newly emerged adult beetles (F_1 population) was recorded at 2, 4 and 6 months after seed treatment.. The vials were observed daily to note the adult emergence starting 20 days after release. The number of adult beetles emerged were recorded on each alternate day. The counting was continued till the complete emergence of F_1 progeny. Finally number of adults emerged in each vial were counted. After complete emergence of F_1 population the vials containing the treated seeds were fumigated with aluminium phosphide tablet for 7 days to prevent the emergence of F_2 population which would hamper recording further observations on seed damage (%), moisture content (%), germination (%), vigour index , weight loss due to infestation (%) and analysis of protein content of infested seeds in due course of time.

3.2.6.4 Moisture content of seed

Prior to use of both seeds as test material, initial moisture content was taken. Similarly moisture content of treated seeds artificially infested by releasing pulse beetles at different storage intervals (1 day, 2, 4 and 6 months after treatment) was also recorded after emergence of F_1 progeny. The moisture content of seeds was determined by oven dry method. The initial weight of aluminium cup was taken and then reweighed with seeds. It was kept in the oven for 1 hour at 130°C. After 1 hour the cup with seeds was removed from oven and weighed. Further, the cup with seeds was kept in the oven for 1 hour and second weight was taken. The process was repeated till three constant weights were obtained. Then the seed moisture content was computed by adopting following formula and expressed in percentage.

$$\text{Moisture content} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

where, M_1 = weight of empty moisture box

M_2 = weight of moisture box with seed before drying

M_3 = Weight of moisture box with seed after drying

3.2.6.5 Seed germination test

Prior to seed treatment, initial germination percentage of pulse seeds was recorded. Further, germination per cent of treated pulse seeds exposed to pulse beetles damage at different storage periods (2, 4 and 6 months after seed treatment) was recorded after emergence of adult beetles (F_1 progeny). Seed germination test was conducted by between paper towel, B.P. method (ISTA, 1985). Fifty seeds in 6 replicates from each treatment and replication wise were randomly counted and placed between moisture paper towel/germination paper and kept inside the germinator at 30°C (Fig. 6 and 7). Observations were taken by counting the number of normal seedling in each replicate. First count was taken 4 days after placing the seeds in between paper towel whereas final count was done 3 days after first count. Germination per cent was calculated by using the following formula.

$$\text{Germination (\%)} = \frac{\text{Total number of normal seedling}}{\text{Total number of seeds}} \times 100$$

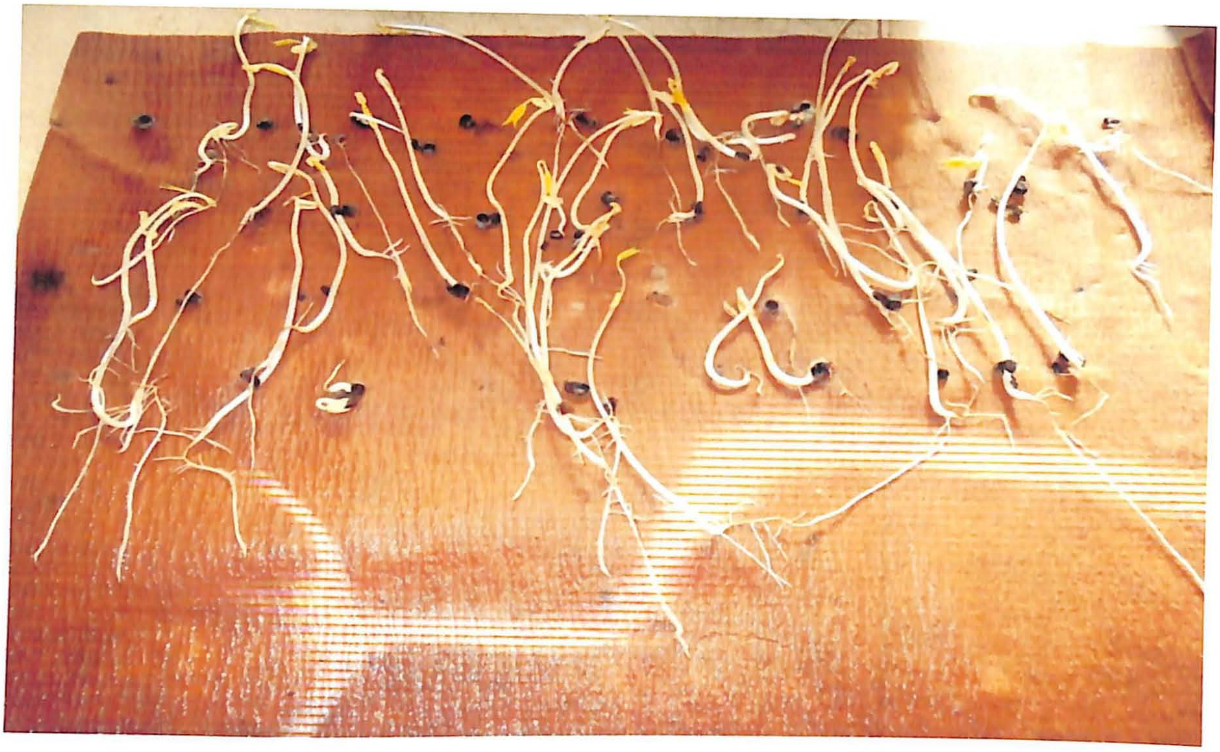


Fig. 6 Effect of insecticidal treatments on germination of black gram seeds (after 6 months storage).

Fig. 7 Effect of insecticidal treatments on germination of green gram seeds (after 6 months storage).

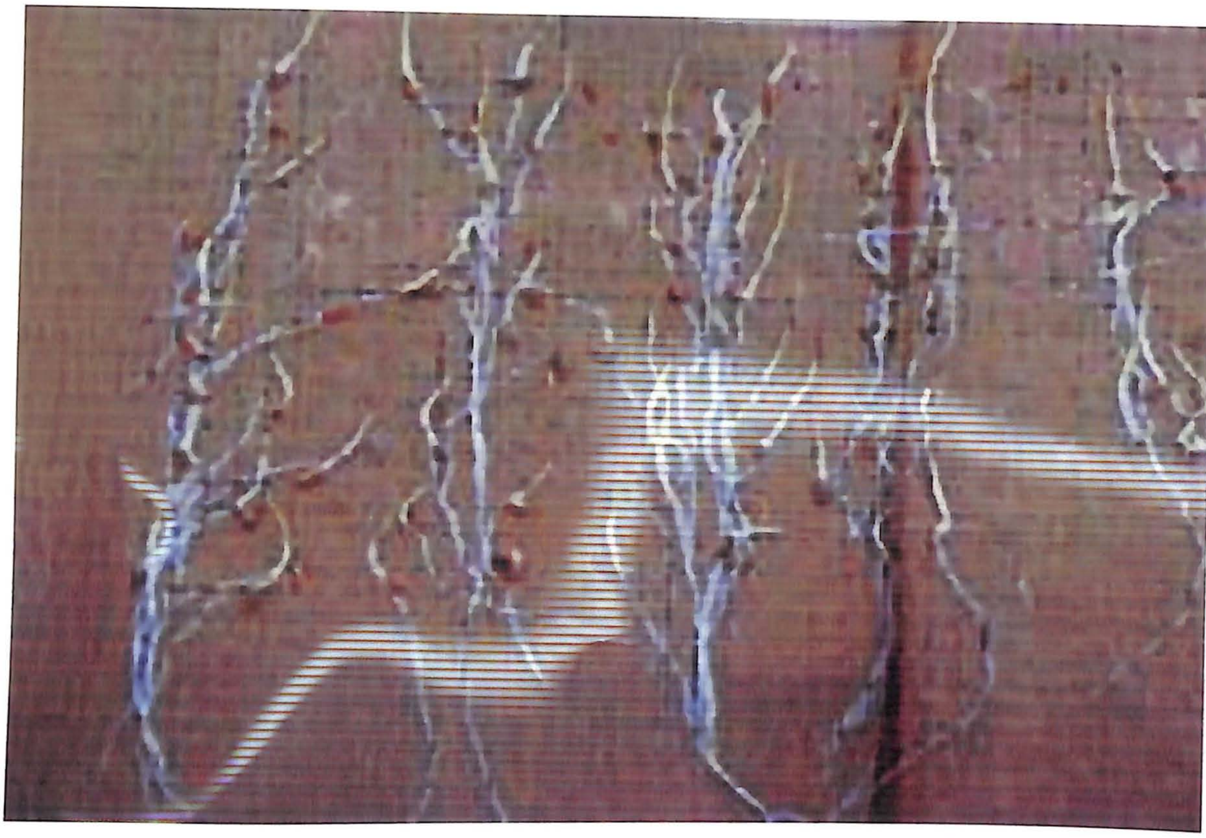


Fig. 8 Effect of botanical treatments on vigour index of black gram seeds (After 6 months storage).

Fig. 9 Effect on botanical treatments on vigour index of green gram seeds(After 6 months storage).

5.2.6.6 Vigour index (VI)

Observations on vigour index of treated pulse seeds exposed to pest infestation as taken after 2, 4 and 6 months of storage. Vigour index was calculated as per Abdul-Baky and Anderson (1973). Length of 10 randomly selected seedlings was measured after taking the germination count at the time of final counting (7 days after germination test) (Fig. 8 and 9). Vigour index was calculated by following formula

Vigour index = Germination (%) x Average length of normal seedlings

3.2.6.7 Seed damage

Observations on percentage seed damage was taken in treated pulse seeds exposed to pulse beetle infestation after 2, 4 and 6 months of storage. The seed damage (%) was determined directly by visual observation of exit holes in 100 number of randomly selected seeds taken from each jar or sample after emergence of F_1 adults (Fig. 10 and 11).

3.2.6.8 Weight loss of infested seeds

The estimation of weight loss (%) of infested seeds was carried out in treated pulse seeds exposed to beetle infestation after 6 months storage period. In each sample number of damaged and undamaged seeds were counted. Further weight of damaged and undamaged seeds were recorded. The per cent weight loss was calculated by count and weight method given by Adams and Schulten (1978) using the following formula

$$\text{Weight loss (\%)} = \frac{UNd - DNu}{U(Nd + Nu)} \times 100$$

Where, U = weight of undamaged seeds

D = Weight of damaged seeds

Nd = Number of damaged seeds

Nu = Number of undamaged seeds

Fig. 10 Seed stock of green gram infested by pulse beetle

Fig. 11 Seed stock of black gram infested by pulse beetle

3.2.6.9 Analysis of protein content of infested seeds

Analysis of protein content in damaged samples (after emergence of F_1 population) of treated pulse seeds due to exposure of pulse beetle after 6 months of storage periods was conducted in Seed physiology laboratory. Prior to seed treatment initial observation on protein content of healthy green gram and black gram seeds was taken.

Protein content was estimated by Lowry's Method developed by Lowry *et al.* (1951). In this method at first green gram seeds damaged by *Callosobruchus chinensis* L. was taken and weighed @ 0.2 g per sample. Then it was macerated by pestle and mortar in 10 ml TCA (10 %) solution. Each sample was transferred to separate centrifuge tube and centrifuged at 5000 rpm for 10 minutes. Supernatant of centrifuge tube was discarded. 10 ml of 1N NaOH was added to tube and mixed well with the helpof glass rod. Again tube was centrifuged at 1000 rpm for 10 minutes. Supernatant was collected for true protein estimation. In the estimation process 0, 0.4, 0.8, 1.2, 1.6 and 2.0 ml of the working standard into a series of test tubes were pipetted out. 0.2 ml of 1N NaOH was added to each test tube and 0.2 ml of sample extract into test tube was pipetted out. The volume to 2 ml in all the test tubes was made up with water. 10 ml of reagent 'C' was added to each tube including the blank, mixed well and allowed stand for 10 minutes. (Reagent 'C'=50 ml of reagent 'A' mixed with 1 ml of reagent 'B'. Reagent A = 2 % NO_2CO_3 in 0.1 N NaOH solution, Reagent B = 0.5 % $CuSO_4$ 5 H_2O in 1 % K-Na-tartarate solution). Then 1 ml reagent D (1 N Folin –Cicalteau reagent) was added, mixed well and incubated at room temperature in the dark for 30 minutes till the blue colour was developed. O.D of samples and standards was noted at 660 nm. A standard graph was drawn and protein content was calculated. Protein content of damaged black gram seeds and healthy black gram, green gram seeds were calculated by this process. Protein content was calculated by following formula.

Protein content = W x O.D value x dilution factor

3.2.7 Statistical analysis

The data generated on different observations were subjected to statistical analysis after suitable transformation wherever necessary as per Gomez and Gomez (1984).

CHAPTER-IV

RESULTS

RESULTS

Experiments were conducted in the Seed Entomology laboratory of Department of Seed Science and Technology, College of Agriculture, OUAT, Bhubaneswar during 2014-15 to determine the effectiveness of some chemical insecticides and botanicals as their seed treatment in green gram and black gram against pulse beetle, *Callosobruchus chinensis* L. infestation during storage under ambient condition. The investigations on residual effect of insecticides and botanicals on mortality, biology of pest, its impact on seed quality parameters, quantitative losses and qualitative(nutritional) changes due to bruchid infestation were carried out by exposing the pests on treated seeds at different time intervals after seed treatment. The data thus obtained pertaining to various experiments were critically analysed and results are incorporated in the present chapter.

4.1 Residual toxicity

4.1.1 Effect of chemical insecticides on mortality of pulse beetle, *Callosobruchus chinensis* L.

4.1.1.1 Green gram

The data pertaining to residual toxicity of insecticides on mortality of pulse beetle in treated green gram seeds are presented in Table 5. The results indicated that complete kill (100 %) was recorded in all the treatments except control at 7 days after releasing (DAR) insects in freshly treated seeds (1 DAST). After 2 and 4 months of seed treatment , highest mortality (95.0 and 87.5 %) was observed in emamectin benzoate followed by deltamethrin (92.5 and 82.5 %) treatment indicating higher residual toxicity against the pest. However, both of them remained at par with respect to percentage mortality of pest. After 6 months of seed treatment, deltamethrin treated green gram seeds registered highest mortality (77.5 %) and remained almost at par with emamectin benzoate treatment (75.5 %). Other two treatments i.e., spinosad and profenofos also showed more or less equally good results and exhibited more than 70.0 % mortality of the pest. Novaluron was found least effective against the pest and recorded minimum mortality (57.5 %).

Table 5. Residual toxicity of insecticides on mortality of *Callosobruchus chinensis* L. in treated green gram seeds at different time intervals after seed treatment during storage

	Treatment	Concentration in ppm (a.i.)	Mortality (%) of pulse beetle at different storage periods (7 DAR)*			
			1 DAST	2 MAST	4 MAST	6 MAST
T_1	Emamectin benzoate (Elpida 5 SG)	2ppm (40.0 mg/kg seed)	100 (89.43)	95.0 (80.49)	87.5 (69.53)	75.5 (60.11)
T_2	Spinosad (Tracer 45 SC)	2 ppm (4.4 mg/kg seed)	100 (89.43)	85.0 (67.50)	77.5 (61.78)	72.5 (58.45)
T_3	Profenofos (Carina 50 EC)	2 ppm (0.004 ml/kg seed)	100 (89.43)	77.5 (61.78)	72.5 (58.45)	72.5 (58.45)
T_4	Novaluron (Rimon 10 EC)	5 ppm (0.05 ml/kg seed)	100 (89.43)	72.5 (58.46)	65.0 (53.78)	57.5 (49.32)
T_5	Deltamethrin (Decis 2.8 EC)	1 ppm (0.04 ml/kg seed)	100 (89.43)	92.5 (76.03)	82.5 (65.47)	77.5 (61.78)
T_6	Untreated control	-	0 (0.57)	0 (0.57)	0 (0.57)	0 (0.57)
	SEm (±)			3.10	1.67	1.53
	CD (0.05)			9.20	4.97	4.54

* Mean of 4 replications, DAR = Days after release,
DAST = Day after seed treatment, MAST = months after seed treatment,
Figures in parentheses are transformed values

4.1.1.2 Black gram

The data as indicated in Table 6 that 100 % cumulative mortality after 7 days of releasing pests was witnessed in all treatments except novaluron (95.0 %) in freshly treated black gram seeds (1 DAST). At 2 and 4 months after seed treatment (MAST), emamectin benzoate treatment registered maximum mortality (92.5 and 87.5 %) of the pest followed by spinosad (87.5 and 85.0 %) in treated seeds during storage. The trend was almost similar at 6 MAST. It was found 72.50 and 67.50 in emamectin benzoate and spinosad treatments respectively and remained at par. All other treatments except novaluron (45.0 %) recorded more than 60.0 % mortality of pest after 6 months of seed treatment in stored black gram. The novaluron consistently proved comparatively less effective as seed protectant period with lower residual toxicity against the pest. The untreated control recorded nil mortality of pest after 6 months of seed treatment.

Table 6. **Residual toxicity of insecticides on mortality of *Callosobruchus chinensis* L. in treated black gram seeds at different storage intervals after seed treatment**

	Treatment	Concentration in ppm (a.i.)	Mortality (%) of pulse beetle at different storage periods (7DAR)*			
			1 DAST	2 MAST	4 MAST	6 MAST
T_1	Emamectin benzoate (Elpida 5 SG)	2ppm (40.0 mg/kg seed)	100 (89.43)	92.5 (76.02)	87.50 (69.53)	72.50 (58.45)
T_2	Spinosad (Tracer 45 SC)	2 ppm (4.4 mg/kg seed)	100 (89.43	87.5 (69.53)	85.0 (67.50)	67.50 (55.28)
T_3	Profenofos (Carina 50 EC)	2 ppm (0.004 ml/kg seed)	100 (89.43)	80 (63.44)	72.50 (58.45)	62.50 (52.28)
T_4	Novaluron (Rimon 10 EC)	5 ppm (0.05 ml/kg seed)	100 (89.43	75.5 (60.12)	65.0 (53.78)	45.0 (42.11)
T_5	Deltamethrin (Decis 2.8 EC)	1 ppm (0.04 ml/kg seed)	100 (89.43	85.0 (67.50)	82.50 (66.84)	65.0 (53.78)
T_6	Untreated control	-	0 (0.57)	0 (0.57)	0 (0.57)	0 (0.57)
	SEm (±)		2.11	2.35	2.17	1.48
	CD (0.05)		6.26	6.99	6.44	4.39

* Mean of 4 replications, DAR = Days after release,
DAST = Day after seed treatment, MAST = months after seed treatment,
Figures in parentheses are transformed values

4.1.2 Effect of botanicals on mortality of pulse beetle, *Callosobruchus chinensis* L.

4.1.2.1 Green gram

The data presented in Table 7 revealed significant differences in the mortality of pulse beetle at 7 days after release when the seeds were treated with different botanicals and exposed to the pest at different time intervals after seed treatment during storage. All the seed treatments showed superiority over control in killing pulse beetle. In freshly treated seed (1 DAST), significantly highest mortality (97.5 %) of bruchid was recorded in seed treated with sweet flag formulation followed by citronella oil (92.5 %). After 2 months of seed treatment, sweet flag formulation treatment killed 85.0 % of the pest population followed by begunia leaf powder (77.5 %). Sweet flag formulation recorded maximum mortality (77.5 and 67.5 %) of the pest followed by citronella oil (72.5 and 65.0 %) after 4 and 6 months of seed treatment and remained statistically at par with each other with respect to corresponding time interval. Begunia leaf powder showed moderate efficacy in killing the pest and recorded 62.5 % mortality at 6 months after seed treatment. Among botanicals, naguari leaf powder proved less effective and registered significantly lowest (45.0 %) mortality after 6 months of seed treatment, whereas no mortality was found in untreated control.

Table 7. Residual toxicity of botanicals on mortality of *Callosobruchus chinensis* L. in treated green gram seeds at different storage intervals after seed treatment

	Treatment	Concentration	Mortality (%) of pulse beetle at different storage periods (7DAR)*			
			1 DAST	2 MAST	4 MAST	6 MAST
T_1	*Vitex negundo* (Begunia leaf powder)	10 g/kg seed	90.0 (71.56)	77.5 (61.78)	67.5 (65.29)	62.5 (52.27)
T_2	*Lantana camera* (Naguari leaf powder)	10 g/kg seed	65.0 (53.80)	57.5 (49.33)	47.5 (43.56)	45.0 (42.11)
T_3	*Murrya koenegii* (Curry leaf powder)	10 g/kg seed	77.5 (61.78)	65.0 (53.78)	55.0 (47.89)	55.0 (47.89)
T_4	*Citronella mucronata* (Citronella oil)	10 ml/kg seed	92.5 (76.02)	72.5 (58.40)	72.5 (58.45)	65.0 (53.78)
T_5	*Acorus calamus* (Sweet flag formulation) 6EC	10 ml/kg seed	97.5 (84.96)	85.0 (67.50)	77.5 (61.77)	67.5 (55.29)
T_6	Untreated control	-	0 (0.57)	0 (0.57)	0 (0.57)	0 (0.57)
	SEm(±)		2.76	1.64	1.45	1.48
	CD (0.05)		8.20	4.88	4.31	4.39

* Mean of 4 replications, DAR = Days after release,
DAST = Day after seed treatment, MAST = months after seed treatment,
Figures in parentheses are transformed values

4.1.2.2 Blackgram

The data showed varying degrees of mortality of pest among botanical treatments at different time intervals after seed treatment during storage (Table 8). Following 7 days exposure period to freshly treated seeds (1DAST), cent per cent mortality was recorded in sweet flag formulation treatment followed by begunia leaf powder (95.0%). The scenario after 2 and 4 months of seed treatment indicated maximum mortality (90.0 % and 77.5 %) in seed treatment with sweet flag formulation followed by begunia leaf powder (85.0 and 75.0 % mortality) and differences remained non-significant. After 6 months stroage period, seed treatment with sweet flag formulation maintained prolonged residual effect and recorded significnatly highest mortality (75.0 %) of pest than begunia leaf powder (65.0%) and citronella oil (62.5 %). The mortality percentage in other treatments of curry and naguari leaf powder was found 57.5 and 45.0 %, respectively after 6 months of seed treatment. Seed treatment with naguari leaf powder had minimum residual effect and resulted least mortality of pest among different botanical seed treatments throughout the period of investigation. Citronella oil treated seeds showed moderate efficacy and withnessed 92.5 % mortality of pest in freshly treated seed which reduced to 62.5 % when bruchids were exposed to treated seeds after 6 months of seed treatment.

Table 8. Residual toxicity of botanicals on mortality of *Callosobruchus chinensis* L. in treated black gram seeds at different storage intervals after seed treatment

Treatment		Concentration	Mortality (%) of pulse beetle at different storage periods (7 DAR)*			
			1 DAST	2 MAST	4 MAST	6 MAST
T_1	*Vitex negundo* (Begunia leaf powder)	10 g/kg seed	95.0 (80.50)	85.0 (67.50)	75.0 (60.12)	65.0 (53.78)
T_2	*Lantana camera* (Naguari leaf powder)	10 g/kg seed	72.5 (58.45)	67.5 (55.28)	55.0 (47.89)	45.0 (42.12)
T_3	*Murrya koenegii* (Curry leaf powder)	10 g/kg seed	85.0 (67.50)	77.5 (61.78)	62.5 (52.28)	57.5 (49.33)
T_4	*Citronella mucronata* (Citronella oil)	10 ml/kg seed	92.5 (76.30)	82.5 (65.47)	72.5 (58.45)	62.5 (52.28)
T_5	*Acorus calamus* (Sweet flag formulation) 6EC	10 ml/kg seed	100.0 (89.43)	90.0 (71.56)	77.5 (61.78)	75.0 (60.12)
T_6	Untreated control	-	0 (0.57)	0 (0.57)	0 (0.57)	0 (0.57)
	SEm (±)		3.02	1.56	1.54	1.58
	CD (0.05)		8.98	4.64	4.58	4.51

* Mean of 4 replications, DAR = Days after release,
DAST = Day after seed treatment, MAST = months after seed treatment,
Figures in parentheses are transformed values

4.2 Fecundity/oviposition

4.2.1 Effect of insecticides on oviposition of *Callosobruchus chinensis* L.in treated pulse seeds

4.2.1.1 Green gram

The pulse beetle laid significantly variable number of eggs in insecticidal treated green gram seeds. Results revealed that fecundity was significantly higher in control than rest of seed treatments (Table 9). After 2 months of seed treatment, less than 5 number of eggs were laid by the pest exposed to emamectin benzoate (1.75), deltamethrin (2.75) and spinosad (3.75) treated seeds. Seed treatments with emamectin benzoate also recorded lowest numer of eggs (3.75) followed by deltamethrin (4.75) after 4 months of storage period and differences were non-significnat. After 6 months of storage period, minimum of eggs were laid (7.25) in deltamethrin treated seeds and did not differ significantly from emamectin benzoate treatment (9.00). Untreated control recorded significantly highest number of eggs (75.50) after 6 months of seed treatment. Novaluron as seed protectant did not show much effect to restrict the egg laying by bruchids.

Table 9. Effect of chemical seed treatments on oviposition of *Callosobruchus chinensis* L. infestation in green gram at 2 month intervals during storage

Treatment		Concentration in ppm (a.i.)	No. of eggs laid/200 seeds*		
			2 MAST	4 MAST	6 MAST
T_1	Emamectin benzoate (Elpida 5 SG)	2ppm (40.0 mg/kg seed)	1.75 (1.49)	3.75 (2.05)	9.00 (3.06)
T_2	Spinosad (Tracer 45 SC)	2 ppm (4.4 mg/kg seed)	3.75 (2.06)	7.00 (2.73)	10.50 (3.31)
T_3	Profenofos (Carina 50 EC)	2 ppm (0.004 ml/kg seed)	5.20 (2.39)	8.50 (2.99)	14.25 (3.83)
T_4	Novaluron (Rimon 10 EC)	5 ppm (0.05 ml/kg seed)	7.25 (2.78)	12.75 (3.63)	26.00 (5.14)
T_5	Deltamethrin (Decis 2.8 EC)	1 ppm (0.04 ml/kg seed)	2.75 (1.80)	4.75 (2.27)	7.25 (2.78)
T_6	Untreated control	-	14.75 (3.89)	42.75 (6.57)	75.50 (8.71)
	SEm (±)		0.09	0.11	0.13
	CD (0.05)		0.26	0.34	0.39
	CV (%)		12.07	12.43	13.23

*Mean of 4 replications, MAST – Months after seed treatment
Figures in parentheses are transformed values

4.2.1.2 Black gram

It is evident from table 10 that minimum number of eggs (3.0) recorded in emamectin benzoate was on par with spinosad (3.25) and deltamethrin (4.0) after 2 months of seed treatment. At 4 MAST (month after seed treatment), spinosad was found superior over other by registering lowest number of eggs (4.25) and did not show significant variation from rest of insecticidal seed treatments (6.50-8.25 eggs) except novaluron (16.25 eggs). The emamectin benzoate treatment suppressed egg laying by bruchids even at 6 MAST in stored black gram where least number of eggs oviposited (8.75 eggs) were at per with that of spinosad (9.75 eggs) and deltamethrin (11.25 eggs). Novaluron seed treatment did not afford much residual toxicity and significantly higher number of eggs were noticed (7.5 to 21.75) among different treatments. Untreated control had however witnessed significantly highest number of eggs (19.0, 58.0 and 82.5 eggs) in black gram seeds at 2, 4 and 6 months after seed treatment respectively, during storage.

Table 10. Effect of insecticides on oviposition of *Callosobruchus chinensis* L. attack in treated black gram seeds at 2 month intervals after seed treatment during storage

Treatment		Concentration in ppm (a.i.)	No. of eggs laid/200 seeds*		
			2 MAST	4 MAST	6 MAST
T_1	Emamectin benzoate (Elpida 5 SG)	2ppm (40.0 mg/kg seed)	3.00 (1.63)	6.50 (2.63)	8.75 (3.03)
T_2	Spinosad (Tracer 45 SC)	2 ppm (4.4 mg/kg seed)	3.25 (1.92)	4.25 (2.17)	9.75 (3.20)
T_3	Profenofos (Carina 50 EC)	2 ppm (0.004 ml/kg seed)	5.75 (2.49)	8.50 (2.98)	18.25 (4.32)
T_4	Novaluron (Rimon 10 EC)	5 ppm (0.05 ml/kg seed)	7.50 (2.82)	16.25 (4.07)	21.75 (4.71)
T_5	Deltamethrin (Decis 2.8 EC)	1 ppm (0.04 ml/kg seed)	4.00 (2.09)	7.25 (2.77)	11.25 (3.41)
T_6	Untreated control	-	19.00 (4.40)	58.00 (6.89)	82.50 (9.11)
	SEm (±)		0.15	0.37	0.14
	CD (0.05)		0.46	1.10	0.42
	CV (%)		19.42	39.34	13.37

*Mean of 4 replications, MAST = Months after seed treatment
Figures in parentheses are transformed values

4.2.2 Effect of botanical seed treatments on oviposition by *Callosobruchus chinensis* L. on treated pulse seeds

4.2.2.1 Green gram

The data as indicated in Table 11 envisaged that sweet flag formulation treatment was most effective in restricting the oviposition by pest in green gram and recorded significantly lowest number of eggs (3.75, 5.75 and 8.0 eggs) when pest was exposed to treated seeds at 2, 4 and 6 months after seed treatment respectively. Next better treatment was begunia leaf powder which registered 6.25 eggs after 2 months of seed treatment and remained on par with citronella oil treatment (7.0 eggs). On the contrary, after 4 and 6 months of seed treatment, citronella oil was found better over others next to sweet flag formulation treatment which recorded 9.75 and 11.50, number of eggs, remained statistically at par with begunia leaf powder (10.75 and 17.0 eggs). Seeds mixed with naguari leaf powder was less effective and stimulated the pest to oviposit significantly more number of eggs (9.75 to 29.0 eggs) among botanicals tested as against 25.50 and 90.50 eggs in untreated control during the period of study.

Table 11. Effect of botanicals on oviposition of *C. chinensis* infestation in treated green gram seeds at 2 month intervals after seed treatment during storage

Treatment		Concentration	No. of eggs laid/200 seeds*		
			2 MAST	4 MAST	6 MAST
T_1	*Vitex negundo* (Begunia leaf powder)	10 g/kg seed	6.25 (2.60)	10.75 (3.35)	17.00 (4.18)
T_2	*Lantana camera* (Naguari leaf powder)	10 g/kg seed	9.75 (3.20)	14.25 (3.83)	29.00 (5.42)
T_3	*Murrya koenegii* (Curry leaf powder)	10 g/kg seed	8.25 (2.95)	13.00 (3.67)	12.50 (3.60)
T_4	*Citronella mucronata* (Citronella oil)	10 ml/kg seed	7.00 (2.73)	9.75 (3.19)	11.50 (3.45)
T_5	*Acorus calamus* (Sweet flag formulation) 6EC	10 ml/kg seed	3.75 (2.06)	5.75 (2.49)	8.00 (2.91)
T_6	Untreated control	-	25.50 (5.08)	65.25 (8.10	90.50 (9.53)
	SEm (±)		0.09	0.12	0.13
	CD (0.05)		0.26	0.35	0.38
	CV (%)		11.27	11.68	11.77

*Mean of 4 replications, MAST = Months after seed treatment
Figures in parentheses are transformed values

4.2.2.2 Black gram

The impact of leaf powders, plant oils and formulations on fecundity of pulse beetle was studied in treated black gram seeds and data are presented in Table

12. The result depicted that minimum eggs (3.25) recorded in seed coated with sweet flag formulation was significantly different from begunia leaf powder (5.75 eggs). citronella oil (7.75 eggs), curry leaf powder (8.25 eggs) and naguari leaf powder (9.0 eggs) after 2 months of seed treatment. At 4 MAST, sweet flag formulation treatment showed lowest number of eggs (5.0 eggs) and differed significantly from other botanical treatments (7.75 to 18.50 eggs). After 6 months of seed treatment when the treated seeds were exposed to bruchid infestation, minimum number eggs oviposited in sweet flag formulation (8.5 eggs) were on par with begunia leaf powder (10.75 eggs). Seeds mixed with curry leaf powder had comparatively better antioviposition effect (8.25-12.50 eggs) than naguari leaf powder (9.75-29.0 eggs). The seeds without botanical treatment encouraged the pest to oviposit significantly highest number of eggs @ 18.50, 61.50 and 82.50 eggs after 2, 4 and 6 months of seed treatment, respectively when infested by bruchids.

Table 12. Effect of botanicals on oviposition of *C. chinensis* L. attack on treated black gram seeds at 2 month intervals during storage

Treatment		Concentration	No. of eggs laid/200 seeds*		
			2 MAST	4 MAST	6 MAST
T_1	*Vitex negundo* (Begunia leaf powder)	10 g/kg seed	5.75 (2.50)	7.75 (2.87)	10.75 (3.33)
T_2	*Lantana camera* (Naguari leaf powder)	10 g/kg seed	9.00 (3.08)	18.50 (4.35)	26.75 (5.21)
T_3	*Murrya koenegii* (Curry leaf powder)	10 g/kg seed	8.25 (2.95)	9.25 (3.11)	15.50 (4.00)
T_4	*Citronella mucronata* (Citronella oil)	10 ml/kg seed	7.75 (2.86)	11.00 (3.38)	12.00 (3.53)
T_5	*Acorus calamus* (Sweet flag formulation) 6EC	10 ml/kg seed	3.25 (1.91)	5.00 (2.34)	8.50 (2.99)
T_6	Untreated control	-	18.50 (4.35)	61.50 (7.87)	82.50 (9.11)
	SEm (±)		0.11	0.10	0.12
	CD (0.05)		0.33	0.30	0.35
	CV (%)		13.46	10.15	11.09

*Mean of 4 replications, MAST= Months after seed treatment
Figures in parentheses are transformed values

4.3 Population build up/ adult emergence of *Collosobruchus chinensis* L.(F_1 Progeny)

4.3.1 Effect of insecticides on adult emergence

4.3.1.1 Green gram

The data on effect of insecticidal treatments on emergence of adult *C. chinensis* (F_1 progeny) from green gram seeds exposed to pulse beetle attack at 2 months interval after seed treatment are presented in Table 13.The findings revealed that minimum adult emergence (1.25 adults) observed in emamectin benzoate treated seeds was significantly different from other treatments (2.50 to 9.0 adults) as against 20.50 number adults emerged in control after 2 months of seed treatment. Similarly at 4 MAST, minimum adult emergence was visualized in deltamethrin treatment (3.25 adults) while rest other treatments recorded 5.25 to 16.0 number of adults. The untreated control witnessed significantly maximum number of emerged adults (54.5). When the treated seeds exposed to bruchids after 6 month storage, least number of adults emerged from emamectin benzoate (7.0 adults) were on par with deltamethrin (8.25 adults) and spinosad treatment (8.75 adults).

Table 13. Effect of insecticides on population build up of *C. chinensis* (L.) in treated green gram seeds at bi-monthly intervals during storage

Treatment		Concentration in ppm(a.i.)	No. of adults (F_1 progeny)*		
			2 MAST	4 MAST	6 MAST
T_1	Emamectin benzoate (Elpida 5 SG)	2ppm (40.0 mg/kg seed)	1.25 (1.29)	5.25 (2.39)	7.00 (2.73)
T_2	Spinosad (Tracer 45 SC)	2 ppm (4.4 mg/kg seed)	2.75 (1.80)	5.75 (2.50)	8.75 (3.03)
T_3	Profenofos (Carina 50 EC)	2 ppm (0.004 ml/kg seed)	9.00 (3.07)	11.75 (3.49)	15.25 (3.96)
T_4	Novaluron (Rimon 10 EC)	5 ppm (0.05 ml/kg seed)	8.25 (2.95)	16.00 (4.06)	32.50 (5.73)
T_5	Deltamethrin (Decis 2.8 EC)	1 ppm (0.04 ml/kg seed)	2.50 (1.72)	3.25 (1.93)	8.25 (2.95)
T_6	Untreated control	-	20.50 (4.57)	54.50 (7.41)	75.50 (8.71)
	SEM (±)		0.10	0.07	0.14
	CD (0.05)		0.29	0.21	0.42
	CV (%)		12.81	8.39	13.44

*Mean of 4 replications, MAST = Months after seed treatment
Figures in parentheses are transformed values

4.3.1.2 Black gram

The data indicated in Table 14 envisaged that after 2 months of seed treatment lowest population build up (F_1) was recorded in emamectin benzoate (2.25) followed by deltamethrin (2.75) treatment, were statistically on par with each other. However, significant differences in population build up were noticed in rest of treatments (3.50 to 9.50 adults) after 4 months of seed treatment during storage. Emamectin benzoate treated seeds registered minimum population build up (3.75) followed by spinosad (4.50) and deltamethrin treatment (5.25) and there was no significant differences among them. After 6 months of storage, the seeds treated with emamectin benzoate showed least population build up (8.25) was on par with deltamethrin treatment (9.75). The population build up in other treatments varied significantly 11.25 adults (spinosad) to 30.25 adults (novaluron) being intermediate population in profenofos (17.0 adults). However significantly maximum population build up (28.50-84.50) adults took place in untreated control during this period by exposing pulse beetle to treated seeds after 2 and 6 months of storage period.

Table 14. Effect of insecticides on population build up of *C. chinensis* (L.) in treated black gram seeds at 2 month intervals during storage

Treatment		Concentration in ppm(a.i.)	No. of adults (F_1 progeny)*		
			2 MAST	4 MAST	6 MAST
T_1	Emamectin benzoate (Elpida 5 SG)	2ppm (40.0 mg/kg seed)	2.25 (1.65)	3.75 (2.06)	8.25 (2.94)
T_2	Spinosad (Tracer 45 SC)	2 ppm (4.4 mg/kg seed)	3.50 (1.99)	4.50 (2.22)	11.25 (3.41)
T_3	Profenofos (Carina 50 EC)	2 ppm (0.004 ml/kg seed)	6.75 (2.68)	9.25 (3.10)	17.00 (4.18)
T_4	Novaluron (Rimon 10 EC)	5 ppm (0.05 ml/kg seed)	9.50 (3.16)	19.25 (4.42)	30.25 (5.53)
T_5	Deltamethrin (Decis 2.8 EC)	1 ppm (0.04 ml/kg seed)	2.75 (1.80)	5.25 (2.39)	9.75 (3.19)
T_6	Untreated control	-	28.50 (5.38)	60.50 (7.80)	84.50 (9.21)
	SEM (±)		0.09	0.15	0.15
	CD (0.05)		0.26	0.44	0.44
	CV (%)		11.83	16.37	14.43

*Mean of 4 replications, MAST =Months after seed treatment
Figures in parentheses are transformed values

4.3.2 Impact of botanicals on adult emergence of *C chinensis* L. (F_1 progeny)

4.3.2.1 Green gram

The data presented in Table 15 indicated significant variation on emergence of F_1 adult population in green gram seeds treated with different plant products. When the treated seeds were infested by bruchids after 2 months of storage period, least number of adult emerged (2.75) from seed treatment with sweet flag formulation remained on par with citronella oil (5.00 adults) but varied distinctly from begunia leaf powder treatment (5.50 adults). On exposure of pulse beetle after 4 months of seed treatment, minimum adults emerged in sweet flag formulation treatment (6.75 adult) was at par with begunia leaf powder (8.50) and differed significantly from citronella oil treatment (9.50 adults). After 6 months of seed treatment, sweet flag formulation treated seeds registered lowest number of emerged adults (7.25) followed by citronella oil (11.0) and begunia leaf powder (14.50). Though curry leaf (9.75-18.50 adults) and naguari leaf powder(12.50 -30.00 adults) witnessed comparatively more number of adults but found significantly lower than untreated control (30.75- 85.25adults) during entire period of investigation.

Table 15. Population buildup of *C. chinensis* L. on green gram seeds at bi-monthly intervals as influenced by botanical treatments during storage

Treatment		Concentration	No. of adults (F_1 progeny) emerged *		
			2 MAST	4 MAST	6 MAST
T_1	*Vitex negundo* (Begunia leaf powder)	10 g/kg seed	5.50 (2.44)	8.50 (2.99)	14.50 (3.85)
T_2	*Lantana camera* (Naguari leaf powder)	10 g/kg seed	12.50 (3.60)	23.00 (4.84)	30.00 (5.48)
T_3	*Murrya koenegii* (Curry leaf powder)	10 g/kg seed	9.75 (3.19)	15.25 (3.97)	18.50 (4.34)
T_4	*Citronella mucronata* (Citronella oil)	10 ml/kg seed	5.00 (2.33)	9.50 (3.16)	11.00 (3.37)
T_5	*Acorus calamus* (Sweet flag formulation)	10 ml/kg seed	2.75 (1.79)	6.75 (2.68)	7.25 (2.76)
T_6	Untreated control	-	30.75 (5.55)	73.00 (8.58)	85.25 (9.28)
	SEM (±)		0.19	0.11	0.22
	CD (0.05)		0.55	0.32	0.66
	CV (%)		21.08	10.27	20.34

*Mean of 4 replications, MAST – Months after seed treatment
Figures in parentheses are transformed values

4.3.2.2 Black gram

The number of F_1 adult population emerged from botanicals treated black gram seeds at different storage intervals after seed treatments are presented in Table 16. The adult population count was significantly higher in untreated control as compared to different botanical treatments. The population ranged significantly between 1.75 (sweet flag formulation) to 10.25 (Naguari leaf powder) in different botanical treatments after 2 months of seed treatment. Seed treatment with begunia leaf powder harboured 4.75 number of adults as compared to 5.75 and 7.75 number of adults in citronella oil and curry leaf powder seed treatments, respectively. After 4 and 6 months of seed treatment, sweet flag formulation recorded significantly lowest number of adults (3.75 and 6.25) respectively followed by begunia leaf powder (7.00 and 12.50) population of adult beetles. Seed treatment with naguari leaf powder recorded 20.50 to 25.75 number of F_1 adults during this period were significantly higher than citronella oil (8.25-14.50 adults) and curry leaf powder treatment (15.25 - 19.25 adults).

Table 16. Population build up of *C. chinensis* on black gram seeds at bimonthly intervals as influenced by botanical treatments during storage

Treatment		Concentration	No. of adults (F_1 progeny) emerged *		
			2 MAST	4 MAST	6 MAST
T_1	*Vitex negundo* (Begunia leaf powder)	10 g/kg seed	4.75 (2.27)	7.00 (2.72)	12.50 (3.59)
T_2	*Lantana camera* (Naguari leaf powder)	10 g/kg seed	10.25 (3.26)	20.50 (4.56)	25.75 (5.10)
T_3	*Murrya koenegii* (Curry leaf powder)	10 g/kg seed	7.75 (2.87)	15.25 (3.95)	19.25 (4.42)
T_4	*Citronella mucronata* (Citronella oil)	10 ml/kg seed	5.75 (2.49)	8.25 (2.94)	14.50 (3.85)
T_5	*Acorus calamus* (Sweet flag formulation)	10 ml/kg seed	1.75 (1.49)	3.75 (2.04)	6.25 (2.56)
T_6	Untreated control	-	22.50 (4.78)	66.25 (8.16)	80.75 (9.01)
	SEM (±)		0.15	0.17	0.23
	CD (0.05)		0.44	0.51	0.69
	CV (%)		17.74	17.57	21.40

*Mean of 4 replications, MAST = Months after seed treatment
Figures in parentheses are transformed values

4.4 Seed infestation by *Callasobruchus chinensis* L.

4.4.1 Bioefficacy of insecticides against *Callasobruchus chinensis* infestation in treated pulse seeds

4.4.1.1 Green gram

The data on relative performance of insecticides against bruchid infestation in treated seeds at different time intervals after seed treatment in stored green gram are indicated in Table 17. There was wide variation in percentage seed infestation among various treatments. After 2 months of seed treatment, both emamectin benzoate and spinosad treatment recorded least damage (0.50) and remained statistically at par with spinosad (0.75 %), profenofos (1.0 %) and novaluron (1.25 %) as compared to 3.50 % seed damage in control. Seed infestation ranged significantly between 0.75 to 2.75 % among different treatments after 4 months storage periods of treated seeds as against 9.0 % in control. Minimum seed damage observed in deltamethrin (0.75 %) remained on par with emamectin benzoate and spinosad (1.00 %) and profenofos (1.50 %) except novaluron which recorded 2.75 % damaged seeds.

Table 17. Relative efficacy of insecticides against *C. chinensis* (L.) infestation in treated green gram seeds at 2 months storage intervals after seed treatment

Treatment		Concentration in ppm(a.i.)	Seed damage (%) *		
			2 MAS	4 MAS	6 MAS
T_1	Emamectin benzoate (Elpida 5 SG)	2ppm (40.0 mg/kg seed)	0.50 (0.96)	1.00 (1.22)	1.00 (1.22)
T_2	Spinosad (Tracer 45 SC)	2 ppm (4.4 mg/kg seed)	0.75 (1.09)	1.00 (1.18)	1.25 (1.31)
T_3	Profenofos (Carina 50 EC)	2 ppm (0.004 ml/kg seed)	1.00 (1.22)	1.50 (1.40)	2.50 (1.72)
T_4	Novaluron (Rimon 10 EC)	5 ppm (0.05 ml/kg seed)	1.25 (1.27)	2.75 (1.79)	3.25 (1.93)
T_5	Deltamethrin (Decis 2.8 EC)	1 ppm (0.04 ml/kg seed)	0.50 (0.96)	0.75 (1.09)	1.25 (1.31)
T_6	Untreated control	-	3.50 (1.99)	9.00 (3.07)	10.75 (3.33)
	SEM (±)		0.13	0.11	0.10
	CD (0.05)		0.39	0.33	0.30
	CV (%)		23.83	18.67	15.98

* mean of 4 replications, MAS = months after storage
Figures in parentheses are transformed values

After 6 months of storage, lowest seed damage was visualized in emamectin benzoate treatment (1.00 %) and no significant differences was found with that of spinosad and deltamethrin treatments (1.25%) seed damage (Figure 12). On the contrary, seed infestation in profenofos (2.5 %) and novaluron (3.25 %) treatments were on par with each other after 6 months of seed treatments in stored green gram.

4.4.1.2 Black gram

The data given in Table 18 showed that all seed protectant insecticides were effective against the pest and resulted in significantly lesser seed damage at all the storage intervals after seed treatment compared with untreated control. After 2 months of storage, minimum seed damage (0.75 %) observed in emamectin benzoate remained at par with spinosad and deltamethrin (1.00 %) and profenofos (1.25 %) seed infestation. The seed treatment with novaluron was least effective in restricting seed damage and recorded 1.75 % infested seeds as against 4.00 % in control. After 4 and 6 months of seed treatment, all the insecticides proved superior in preventing seed damage by bruchids when compared to untreated check. After 4 months of storage period, emamectin benzoate treatment recorded minimum seed damage (1.25 %) followed by spinosad (1.50 %) and deltamethrin (1.75 %) and remained at par with one another. The spinosad treatment registered lowest damage (1.50 %) followed by emamectin benzoate and deltamethrin, treatments (2.00 % each) seed infestation after 6 months of storage period (Fig. 12). The percentage damage in these 3 treatments were statistically on par. The profenofos (2.50 and 3.75 %) provided better protection for stored black gram seeds from attack of bruchids than that of novaluron (3.25 and 4.50 %) after 4 and 6 months of seed treatment, respectively.

Table 18. Relative efficacy of insecticides against *C. chinensis* (L.) infestation in treated black gram at 2 month storage intervals after seed treatment

Treatment		Concentration in ppm(a.i.)	Seed damage (%) *		
			2 MAS	4 MAS	6 MAS
T_1	Emamectin benzoate (Elpida 5 SG)	2ppm (40.0 mg/kg seed)	0.75 (1.09)	1.25 (1.31)	2.00 (1.58)
T_2	Spinosad (Tracer 45 SC)	2 ppm (4.4 mg/kg seed)	1.00 (1.22)	1.50 (1.40)	1.50 (1.40)
T_3	Profenofos (Carina 50 EC)	2 ppm (0.004 ml/kg seed)	1.25 (1.31)	2.50 (1.72)	3.75 (2.06)
T_4	Novaluron (Rimon 10 EC)	5 ppm (0.05 ml/kg seed)	1.75 (1.47)	3.25 (1.92)	4.50 (2.22)
T_5	Deltamethrin (Decis 2.8 EC)	1 ppm (0.04 ml/kg seed)	1.00 (1.22)	1.75 (1.49)	2.00 (1.56)
T_6	Untreated control	-	4.00 (2.11)	10.25 (3.27)	12.50 (3.60)
	SEM (±)		0.09	0.10	0.11
	CD (0.05)		0.26	0.30	0.33
	CV (%)		16.64	14.59	15.19

* mean of 4 replications, MAS= Months after storage

Figures in parentheses are transformed values

4.4.2 Bioefficacy of botanicals seed protectants against pulse beetle

4.4.2.1 Green gram

It is evident from Table 19 that maximum protection to treated seeds in terms of reduction of seed damage by bruchids was provided by sweet flag formulation and citronella oil (1.25 % damaged seeds), begunia leaf powder (1.50 %), while curry leaf (2.00 % seed damage) and naguari leaf (2.25 %) were next in order of effectiveness after 4 months of storage period. The untreated control suffered significantly maximum seed damage (4.50 %) during this period.After 4 months storage, least seed infestation was observed in sweet flag formulation treatment (1.75 %) was at par with begunia leaf powder and citronella oil treatment(2.25 %) and curry leaf powder (2.50 %). Naguari leaf with 3.75 % seed damage was least effective but significantly superior to control (10.75 % seed damage). Sweet flag formulation (2.25 % damage), citronella oil and begunia leaf powder treatments (2.50 %) were the best and equally effective in minimizing the seed damage at 6 months after seed treatment during storage(Figure 13). The curry leaf powder (4.25 %) and naguari leaf powder treatment (5.25 %) recorded maximum damage among botanicals and remained at par as compared to significantly maximum damage seeds (13.00 %) in control treatment.

Table 19. Comparative performance of botanicals against *C. chinensis* L. damage in treated green gram at 2 month intervals after seed treatment during storage

Treatment		Concentration	Seed infestation (%) *		
			2 MAS	4 MAS	6 MAS
T_1	*Vitex negundo* (Begunia leaf powder)	10 g/kg seed	1.50 (1.40)	2.25 (1.63)	2.50 (1.70)
T_2	*Lantana camera* (Naguari leaf powder)	10 g/kg seed	2.25 (1.65)	3.75 (2.05)	5.25 (2.39)
T_3	*Murrya koenegii* (Curry leaf powder)	10 g/kg seed	2.00 (1.56)	2.50 (1.70)	4.25 (2.17)
T_4	*Citronella mucronata* (Citronella oil)	10 ml/kg seed	1.25 (1.31)	2.25 (1.65)	2.50 (1.72)
T_5	*Acorus calamus* (Sweet flag formulation)	10 ml/kg seed	1.25 (1.31)	1.75 (1.49)	2.25 (1.63)
T_6	Untreated control	-	4.50 (2.23)	10.75 (3.35)	13.00 (3.66)
	SEM (±)		0.09	0.12	0.13
	CD (0.05)		0.26	0.36	0.39
	CV (%)		15.01	17.57	18.41

* Mean of 4 replications , MAS = Months after storage
Figures in parentheses are transformed values

4.2.2 Black gram

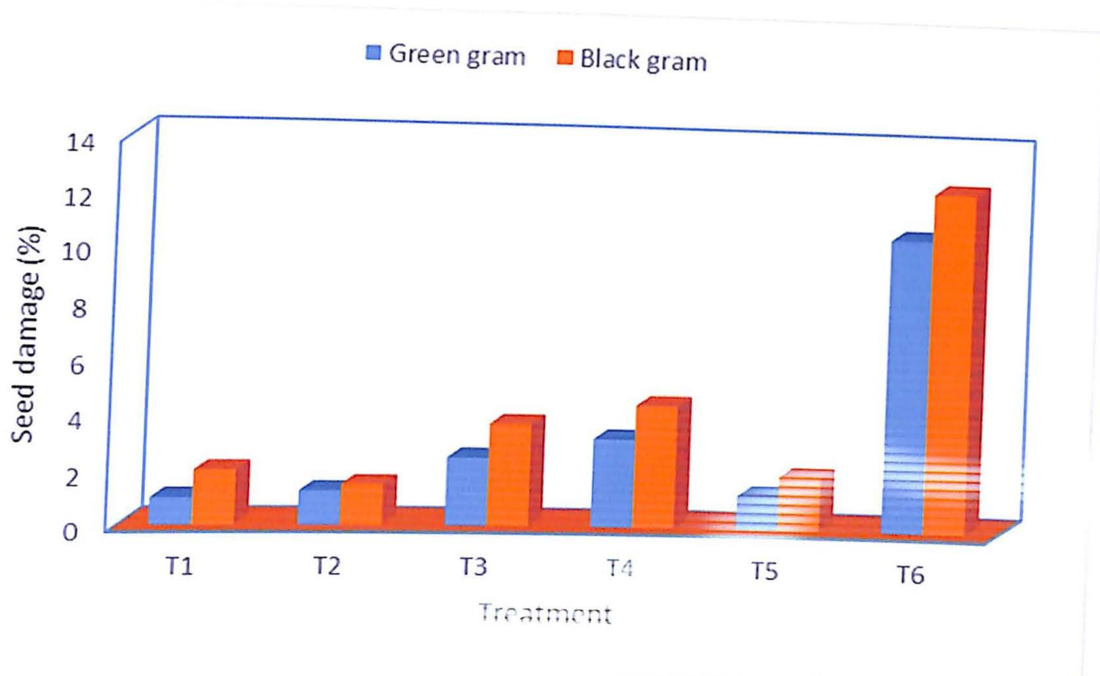
It is indicated from the Table 20 that all the botanical treatments were significantly superior over untreated control in reducing the seed damage recorded at 2 months storage intervals after treatment. Among the control strategies tested against the pest, black gram seeds treated with sweet flag formulation suffered minimum seed damage (0.50 %) was at par with begunia leaf poswer (0.75 %) and citronella oil (1.00 %). The seed treatment with curry and naguari leaf powder on the other hand recorded 1.50 and 1.75 % damaged seeds, respectively as against 4.25 % in control. After 4 months storage period the degree of infestation varied from 1.25 to 8.50 % among different treatments. Sweet flag formulation and begunia leaf powder treatments were most effective and recorded minimum seed damage (1.25 %) remained at par with citronella oil (1.50 %) and curry leaf powder treatment (1.75

%). Seeds mixed with naguari leaf powder was least effective and produced 3.25 % damaged seeds as compared to maximum seed damage in control (8.50 %). Lowest seed damage was noted in begunia leaf powder (1.50 %) followed by sweet flag formulation (1.75 %) and citronella oil (2.25 %) after 6 months of treatment and they were on par with one another Seed treatment with curry and naguari leaf powder were comparatively less effective in checking the pest infestation with 3.00 and 3.75 % damaged seeds as compared to 11.50 % in untreated control(Figure 13).

Table 20. Comparative performance of botanicals against *C. chinensis* L. infestation in treated black gram at 2 months storage intervals after seed treatment

	Treatment	Concentration	Seed infestation (%) *		
			2 MAS	4 MAS	6 MAS
T_1	*Vitex negundo* (Begunia leaf powder)	10 g/kg seed	0.75 (1.09)	1.25 (1.31)	1.50 (1.40)
T_2	*Lantana camera* (Naguari leaf powder)	10 g/kg seed	1.75 (1.49)	3.25 (1.93)	3.75 (2.06)
T_3	*Murrya koenegii* (Curry leaf powder)	10 g/kg seed	1.50 (1.40)	1.75 (1.49)	3.00 (1.86)
T_4	*Citronella mucronata* (Citronella oil)	10 ml/kg seed	1.00 (1.22)	1.50 (1.40)	2.25 (1.65)
T_5	*Acorus calamus* (Sweet flag formulation)	10 ml/kg seed	0.50 (0.96)	1.25 (1.31)	1.75 (1.49)
T_6	Untreated control	-	4.25 (2.17)	8.50 (2.99)	11.50 (3.46)
	SEM (±)		0.09	0.09	0.09
	CD (0.05)		0.26	0.26	0.26
	CV (%)		16.84	13.13	12.29

*Mean of 4 replications , MAS = Months after storage
Figures in parentheses are transformed values



'ig. 12 **Effect of insecticides against *Callosobruchus chinensis* L. infestationin treated green gram and black gram after 6 months of strage**

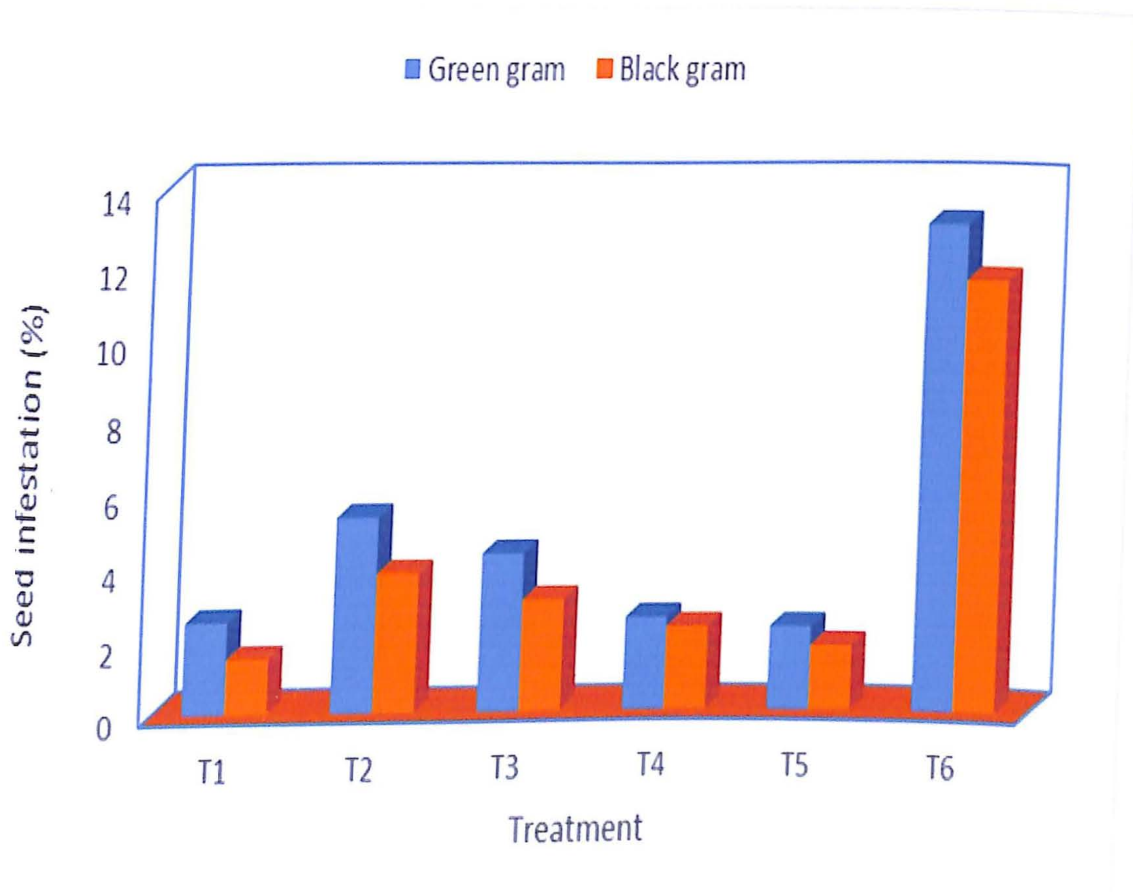


Fig. 13 Effect of botanicals against *Callosobruchus chinensis* L. infestationin treated green gram and black gram after 6 months of strage

4.5 Storability of pulse seeds

4.5.1 Effect of insecticides on quality parameters of treated seeds against bruchids, *C chinensis* L.

4.5.1.1 Green gram

The reduction in the germination and vigour of green gram seeds due to bruchid infestation up to 6 months of storage after seed treatment presented in Table 21. Initial seed germination was 95.0 %, which declined to 86.50 % in control after 2 months of seed treatment. Seeds treated with deltamethrin (93.25 %), emamectin benzoate (93.00 %, spinosad (92.5 %) and profenofos (91.5 %) had significantly higher germination than novaluron (91.1 %). All the treatments including untreated control expressed germination values above IMSCS i.e., more than 75 % after 4 months of seed treatment. However, after 6 months of seed treatment, emamectin benzoate (86.75 %) and deltamethrin (85.0 %) recorded significantly higher germination than rest of treatments. In all the treatments except novaluron (78.0%) and untreated control (73.0 %) showed significantly good germination percentage. The initial seedling vigour index (4475) reduced to 3493 in untreated control after 2 months of seed treatment. All the treatments were superior over control with significantly higher vigour index in emamectin benzoate (4278) and deltamethrin (4124) followed by spinosad (4025), profenofos (3921) and novaluron (3867). Similar trend was noticed after 4 months of treatment. But at 6 MAST maximum vigour index was recorded in deltamethrin (2122) followed by emamectin benzoate (2094) as against 1541 in control. The initial moisture content of seed was 9.0 % and varied 9.1 % (emamectin benzoate) to 10.0 % (control) at 2 MAST. The moisture content of treated seeds slightly increased during storage and varied between 10.0 % (emamectin benzoate) to 11.0 % (control) on exposure of treated seeds to pulse beetle attack after 6 months of seed treatment.

4.5.1.2 Black gram

The effect of chemical treatments on germination per cent, vigour index and moisture content of black gram seeds as influenced by pulse beetle infestation at different storage intervals after seed treatment are presented in Table 22. Germination per cent varied significantly between 85.0 % (control) to 92.75 % (emamectin benzoate) in different treatments at 2 MAST during storage. Initial germination per cent prior to seed

Table 21. Effect of chemical insecticides on storability of green gram seeds against *C. chinensis* L. infestation at 2 month intervals after seed treatment

	Treatment	Concentration in ppm(a.i.)	2 MAST*			4 MAST*			6 MAST *		
			M (%)	G(%)	VI	M (%)	G (%)	VI	M (%)	G(%)	VI
T_1	Emamectin benzoate (Elpida 5 SG)	2ppm (40.0 mg/kg seed)	9.1	93.00 (9.64)	4278.26	10.0	90.50 (9.51)	3555.30	10.0	86.75 (9.31)	2094.65
T_2	Spinosad (Tracer 45 SC)	2 ppm (4.4 mg/kg seed)	9.5	92.50 (9.61)	4025.20	10.2	88.50 (9.41)	3356.20	10.4	83.50 (9.13)	1948.60
T_3	Profenofos (Carina 50 EC)	2 ppm (0.004 ml/kg seed)	9.5	91.50 (9.56)	3921.22	10.3	87.75 (9.37)	3384.40	10.4	82.25 (9.06)	1921.10
T_4	Novaluron (Rimon 10 EC)	5 ppm (0.05 ml/kg seed)	9.7	91.00 (9.54)	3867.60	10.8	85.25 (9.23)	3051.10	11.0	78.0 (8.83)	1793.42
T_5	Deltamethrin (Decis 2.8 EC)	1 ppm (0.04 ml/kg seed)	9.2	93.25 (9.65)	4124.86	9.9	91.50 (9.56)	3527.20	10.1	85.0 (9.22)	2122.30
T_6	Untreated control		10.0	86.50 (9.30)	3493.51	11.2	78.00 (8.83)	2669.25	11.6	73.0 (8.54)	1541.30
	SEM (±)		0.11	0.03	52.81	0.11	0.04	23.00	0.09	0.05	28.70
	CD (0.05)		0.33	0.09	156.88	0.33	0.13	68.33	0.26	0.15	76.35
	CV (%)		7.25	9.71	168.01	7.31	2.99	80.61	5.32	3.16	117.81

* Mean of 4 replications , MAST = Months after seed treatment, M= Moisture, G= Germination, VI= Vigour index
Figures in parentheses are transformed values

Table 22. Effect of chemical insecticides on storability of black gram seeds against *C. chinensis* L. infestation at 2 months intervals after seed treatment

	Treatment	Concentration in ppm(a.i.)	2 MAST*			4 MAST*			6 MAST *		
			M (%)	G(%)	VI	M (%)	G (%)	VI	M (%)	G(%)	VI
T_1	Emamectin benzoate (Elpida 5 SG)	2ppm (40.0 mg/kg seed)	9.3	92.75 (9.65)	3748.45	10.2	90.00 (9.51)	3464.30	10.1	85.50 (9.27)	2020.27
T_2	Spinosad (Tracer 45 SC)	2 ppm (4.4 mg/kg seed)	9.1	92.00 (9.61)	3689.60	10.2	87.50 (9.34)	3253.85	10.3	83.75 (9.18)	1974.00
T_3	Profenofos (Carina 50 EC)	2 ppm (0.004 ml/kg seed)	9.5	89.50 (9.49)	3308.70	10.5	85.00 (9.24)	3197.67	10.7	81.25 (9.04)	1811.77
T_4	Novaluron (Rimon 10 EC)	5 ppm (0.05 ml/kg seed)	9.8	88.00 (9.41)	3110.52	10.9	82.25 (9.10)	2885.17	11.0	77.50 (8.83)	1524.60
T_5	Deltamethrin (Decis 2.8 EC)	1 ppm (0.04 ml/kg seed)	9.5	90.50 (9.54)	3495.45	10.4	86.00 (9.30)	3450.90	10.5	81.75 (9.07)	1865.70
T_6	Untreated control		10.2	85.00 (9.25)	2770.32	11.6	78.50 (8.89)	2511.00	11.9	72.50 (8.55)	1292.20
	SEM (±)		0.09	0.04	38.22	0.09	0.05	35.00	0.13	0.05	29.41
	CD (0.05)		0.27	0.13	113.55	0.26	0.15	103.97	0.39	0.15	87.38
	CV (%)		5.70	2.96	132.00	5.41	3.55	125.17	8.07	3.65	140.70

* Mean of 4 replications , MAST = Months after seed treatment, M= Moisture, G= Germination. VI= Vigour index
Figures in parentheses are transformed values

treatment was recorded 94.0 %. All the treatments expressed good germination values (82.25 -90.00 %) except untreated control (78.50 %) after 4 months of storage in treated seed. After 6 months of seed treatment, all the treatments registered good germination of seed except nuvaluron (77.50 %) and untreated control (72.50 %). Unlike germination similar response in variation of vigour index among treatments and reduction in vigour index after different storage intervals after seed treatment was also observed. Initial vigour index before seed treatment was 3890 reduced to 2770 in control at 2 MAST during storage. The vigour index was significantly higher in emamectin benzoate (3748) and spinosad (3689) than rest of others. After 4 months of seed treatment, maximum vigour index was noticed in emamectin benzoate (3464) followed by deltamethrin (3450) differed significantly from others (2511 -3253). The vigour index drastically reduced and varied from 1292 (control) to 2020 (emamectin benzoate) in various treatments after 6 months storage period of treated seeds. The moisture content prior to seed treatment was 8.9 % and reached at a level of 10.2, 11.6 and 11.9 % in untreated control after 2, 4 and 6 months of seed treatments respectively due to bruchid infestation.

4.5.2 Seed quality attributes of pulse seeds as influenced by botanical treatments against bruchid

4.5.2.1 Green gram

The germination per cent varied significantly between 82.0 to 90.0 per cent among different treatments at 2 MAST (Table 23). After 4 months of storage, all the treatments except naguari leaf powder (78.0 %) and untreated control (75.0 %) showed germination count above 80.0 % in treated seeds. After 6 months of seed treatment, significantly maximum germination count was recorded in sweet flag formulation (82.0 %) remained at par with citronella oil (81.0 %). Rest other treatments except control (67.0 %) expressed germination count above IMSCS i.e., 75 per cent. The vigour index in various treatments followed same trend as germination count. The vigour index prior to seed treatment was 3810 declined to 2696 in control after 2 months of seed treatment.

Among treatments, higher values of vigour index was noted in sweet flag formulation, citronella oil and begunia leaf powder treated seeds than others during the period of study up to 6 months after seed treatment. It was maximum in sweet flag formulation (2081) followed by begunia leaf powder (1953) and citronella oil treatment (1910) after 6 months of seed treatment. The moisture content after 2 months of seed treatment varied between 9.7 to 10.8 per cent in different treatments and gradually increased up to 6 months of storage. The botanicals treated green gram seeds recorded minimum moisture content in sweet flag formulation and citronella oil treatment (10.4 %) and significantly maximum in untreated control (12.4 %).

4.5.2.2 Black gram

It is clear from data presented in Table 24 that the seed germination was significantly higher in sweet flag formulation and begunia leaf powder treatment (92.0 %) than rest treatments being minimum in control (84.0 %) after 2 months of seed treatment. After 4 months storage, the treated seeds recorded the germination count above IMSC i.e., more than 75.0 % ranged significantly between 77.0 to 89.0 % among treatments. The treated seeds showed germination values above 75.0 % in all the treatments except control (71.0 %) even after 6 months of storage. The vigour index gradually reduced among various treatments and varied between 3415 (control) to 4178 (sweet flag formulation) after 2 months of treatment. Which came down and varied from 1419 (control) to 2072 (sweet flag formulation) after 6 months storage in treated seeds. Moisture content after 2 months of storage period ranged between 9.6 % to 10.6 % and it was recorded between 10.2 to 12.2 % among different treatments after 6 months of storage.

Table 23. Impact of botanical insecticides on seed quality attributes in green gram exposed to *C. chinensis* L.attack at 2 month intervals after seed treatment during storage

Treatment		Concentration	2 MAST*			4 MAST*			6 MAST *		
			M (%)	G(%)	VI	M (%)	G (%)	VI	M (%)	G(%)	VI
T_1	*Vitex negundo* (Begunia leaf powder)	10 g/kg seed	10.2	88.0 (9.41)	3400.52	10.6	84.0 (9.19)	3364.12	10.8	78.0 (8.86)	1953.40
T_2	*Lantana camera* (Naguari leaf powder)	10 g/kg seed	10.5	86.0 (9.30)	3014.18	10.9	78.0 (8.86)	2795.97	11.1	72.0 (8.51)	1475.90
T_3	*Murrya koenegii* (Curry leaf powder)	10 g/kg seed	10.2	88.0 (9.41)	3209.45	10.6	82.0 (9.08)	3106.22	10.8	78.0 (8.86)	1731.27
T_4	*Citronella mucronata* (Citronella oil)	10 ml/kg seed	9.8	90.0 (9.51)	8559.67	10.4	84.0 (9.19)	3371.77	10.4	81.0 (9.03)	1910.07
T_5	*Acorus calamus* (Sweet flag formulation)	10 ml/kg seed	9.7	90.0 (9.51)	3641.80	10.3	85.0 (9.25)	3163.47	10.4	82.0 (9.08)	2081.70
T_6	Untreated control	-	10.8	82.0 (9.08)	2696.60	12.0	75.0 (8.69)	2439.05	12.4	67.0 (8.21)	1261.80
	SEM (±)		0.10	0.05	37.46	0.13	0.06	34.61	0.13	0.08	30.96
	CD (0.05)		0.31	0.16	111.29	0.39	0.17	102.83	0.39	0.24	91.98
	CV (%)		6.52	3.69	131.35	8.30	3.92	125.56	8.23	5.57	148.64

* Mean of 4 replications , MAST = Months after seed treatment, M= Moisture, G= Germination, VI= Vigour index
Figures in parentheses are transformed values

Table 24. Impact of botanical insecticides on seed quality parameters in black gram artificially infested by *C. chinensis* L. attack at 2 months storage intervals after seed treatment

Treatment		Concentration	2 MAST*			4 MAST*			6 MAST *		
			M (%)	G(%)	VI	M (%)	G (%)	VI	M (%)	G(%)	VI
T_1	*Vitex negundo* (Begunia leaf powder)	10 g/kg seed	9.6	92.0 (9.62)	4046.32	10.0	89.0 (9.46)	3335.00	10.2	84.0 (9.19)	1995.70
T_2	*Lantana camera* (Naguari leaf powder)	10 g/kg seed	10.3	89.0 (9.46)	3789.57	10.8	85.0 (9.25)	2854.90	11.0	75.0 (8.69)	1712.10
T_3	*Murrya koenegii* (Curry leaf powder)	10 g/kg seed	9.9	91.0 (9.56)	3826.12	10.4	87.0 (9.35)	3235.55	10.4	82.0 (9.08)	1820.87
T_4	*Citronella mucronata* (Citronella oil)	10 ml/kg seed	9.8	91.0 (9.56)	3941.50	10.3	87.0 (9.35)	3164.37	10.4	82.0 (9.08)	1852.97
T_5	*Acorus calamus* (Sweet flag formulation)	10 ml/kg seed	9.6	92.0 (9.62)	4178.05	10.1	88.0 (9.41)	3360.35	10.2	85.0 (9.25)	2072.20
T_6	Untreated control	-	10.6	84.0 (9.19)	3415.65	11.5	77.0 (8.80)	2458.95	12.2	71.0 (8.45)	1419.22
	SEM (±)		0.10	0.03	55.61	0.09	0.05	25.13	0.12	0.05	29.31
	CD (0.05)		0.30	0.10	165.20	0.27	0.15	74.65	0.36	0.15	87.07
	CV (%)		6.76	2.29	178.86	5.34	3.46	90.72	7.75	3.52	137.70

* Mean of 4 replications , MAST = Months after seed treatment, M= Moisture, G= Germination, VI= Vigour index
Figures in parentheses are transformed values

4.6 Qualitative and quantitative changes of treated pulse seeds due to bruchid infestation after 6 months of storage.

4.6.1 Effect of insecticides on percentage weight loss and protein content of treated pulse seeds

4.6.1.1 Green gram

The data on per cent weight loss and protein content of green gram seeds exposed to bruchid attack after 6 months of storage period are presented in Table 25. Initial protein content prior to seed treatment was 12.68 %. It was observed that the seed weight loss (%) due to infestation after 6 months of treatment was significantly minimum in emamectin benzoate (0.22 %) remained on par with deltamethrin (0.24 %0 and spinosad (0.28%) as against maximum in control (2.82 %). There was great variation in protein content of treated seeds infested by bruchids after 6 months of storage period. The protein composition varied significantly in different treatments being minimum in emamectain benzoate (13.44%) was at par with deltamethrin (13.75 %) and maximum in control (18.44 %). The protein content in other treatments ranged between 13.94 to 16.63 per cent (Figure 14 and 15).

Table 25. Percentage weight loss and protein content of insecticide treated green gram seeds exposed to *C. chinensis* L. attack after 6 months storage period

Treatment		Concentration in ppm (a.i.)	6 MAS*	
			Weight loss (%)	Protein content (%)
T_1	Emamectin benzoate (Elpida 5 SG)	2ppm (40.0 mg/kg seed)	0.22 (0.85)	13.14
T_2	Spinosad (Tracer 45 SC)	2 ppm (4.4 mg/kg seed)	0.28 (0.88)	13.94
T_3	Profenofos (Carina 50 EC)	2 ppm (0.004 ml/kg seed)	0.37 (0.93)	15.44
T_4	Novaluron (Rimon 10 EC)	5 ppm (0.05 ml/kg seed)	0.75 (1.11)	16.63
T_5	Deltamethrin (Decis 2.8 EC)	1 ppm (0.04 ml/kg seed)	0.24 (0.86)	13.75
T_6	Untreated control	-	2.82 (1.82)	18.44
	SEM (±)		0.009	0.15
	CD (0.05)		0.03	0.44
	CV (%)		1.61	7.13

* Mean of 4 replications, MAS = Months after storage
Figures in parentheses are transformed values

4.6.1.2 Black gram

It is evident from Table 26 that the emamectin benzoate recorded significantly minimum seed weight loss (0.33 %) followed by spinosad (0.39 %) as compared to 4.07 % in untreated control. Among treated seeds, novaluron treatment registered significantly maximum weight loss (1.08%) when infested by bruchids after 6 months of treatment in stored black gram. Protein content of seeds was significantly lowest in emamectin benzoate (12.96 %) followed by spinosad (13.90%). The significantly highest protein content (19.84 %) was estimated in untreated control. The protein content in other treatments ranged between 14.52 to 17.28 per cent (Figure 14 and 15).

Table 26. Effect of chemical insecticides on Percentage weight loss and protein content in treated black gram seeds exposed to *C. chinensis* L.attack after 6 months storage period

Treatment		Concentration in ppm(a.i.)	6 MAS*	
			Weight loss (%)	Protein content (%)
T_1	Emamectin benzoate (Elpida 5 SG)	2ppm (40.0 mg/kg seed)	0.33 (0.91)	12.96
T_2	Spinosad (Tracer 45 SC)	2 ppm (4.4 mg/kg seed)	0.39 (0.94)	13.90
T_3	Profenofos (Carina 50 EC)	2 ppm (0.004 ml/kg seed)	0.83 (1.15)	16.99
T_4	Novaluron (Rimon 10 EC)	5 ppm (0.05 ml/kg seed)	1.08 (1.25)	17.28
T_5	Deltamethrin (Decis 2.8 EC)	1 ppm (0.04 ml/kg seed)	0.46 (0.98)	14.52
T_6	Untreated control	-	4.07 (2.14)	19.84
	SEM (±)		0.009	0.13
	CD (0.05)		0.3	0.39
	CV (%)		1.56	6.61

* Mean of 4 replications, MAS = Months after storage
Figures in parentheses are transformed values

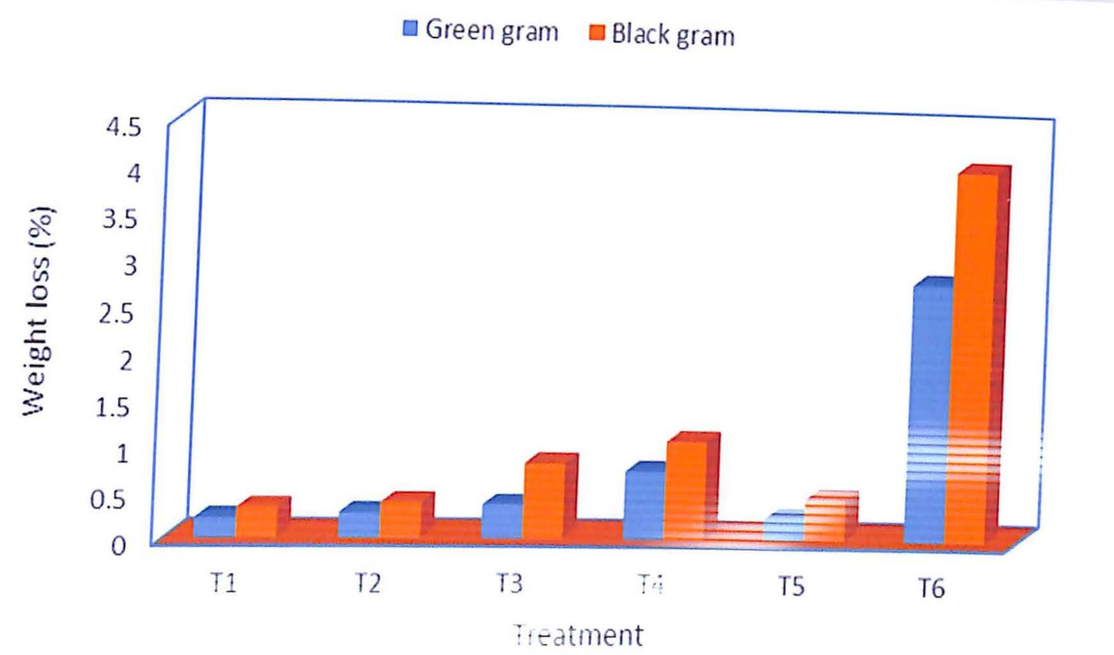


Fig. 14. Percentage weight loss of insecticide treated green gram and black gram seeds exposed to *Callosobruchus chinensis* L. after 6 months of storage

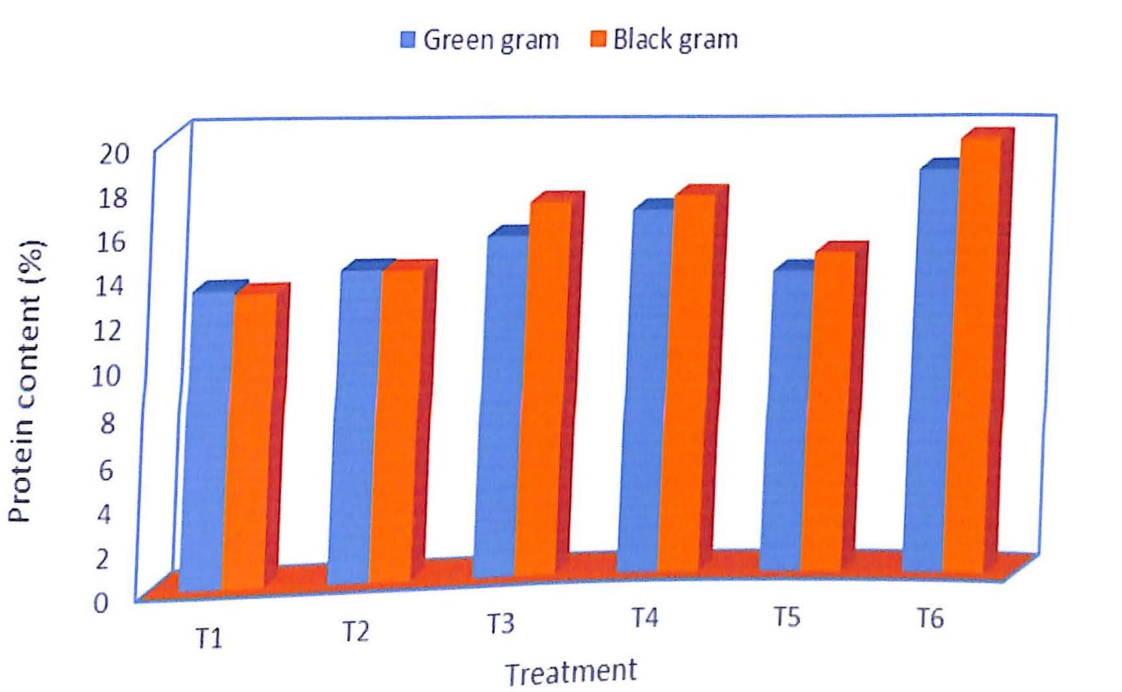


Fig. 15. Protein content of insecticide treated green gram and black gram seeds exposed to *Callosobruchus chinensis* L. after 6 months of storage

4.6.2 Percentage weight loss and protein content of pulse seeds as influenced by botanical treatments

4.6.2.1 Green gram

The results evidenced that minimum weight loss of 0.38 % took place in sweet flag formulation followed by citronella oil (0.55 %) and begunia leaf powder (0.63 %) whereas significantly maximum weight loss (3.86 %) was estimated in untreated control (Table 27). The protein content was calculated lowest in sweet flag formulation (13.96 %) followed by citronella oil (15.12 %) and begunia leaf powder (15.48 %) as against highest in control (19.75 %). The protein content of seeds in citronella oil and begonia leaf powder treatments remained at par with each other (Figures 16 and 17).

Table 27. Percentage weight loss and protein content of botanicals treated green gram seeds exposed to *C. chinensis* L. attack after 6 months storage period

Treatment		Concentration	6 MAS*	
			Weight loss (%)	Protein content (%)
T_1	*Vitex negundo* (Begunia leaf powder)	10 g/kg seed	0.63 (1.06)	15.48
T_2	*Lantana camera* (Naguari leaf powder)	10 g/kg seed	1.05 (1.24)	17.88
T_3	*Murrya koenegii* (Curry leaf powder)	10 g/kg seed	0.71 (1.10)	16.64
T_4	*Citronella mucronata* (Citronella oil)	10 ml/kg seed	0.55 (1.03)	15.12
T_5	*Acorus calamus* (Sweet flag formulation)	10 ml/kg seed	0.38 (0.94)	13.96
T_6	Untreated control	-	3.86 (2.09)	19.75
	SEM (±)		0.009	0.14
	CD (0.05)		0.03	0.42
	CV (%)		1.64	6.96

* Mean of 4 replications, MAS =Months after storage
Figures in parentheses are transformed values

4.6.2.2 Black gram

The data mentioned in Table 28 envisaged great variation in weight loss (%) and protein content (%) of black gram seeds in different treatments due to their differential residual toxicity to bruchid invasion (Figures 16 and 17). Sweet flag formulation recorded minimum weight loss (0.35 %) and protein content (13.25 %) after 6 months of treatment due to bruchid attack followed by begunia leaf powder (0.40 % weight loss and 13.51 % protein content). The untreated control recorded maximum weight loss and protein content of 3.55 and 18.63 per cent, respectively.

Table 28. Impact of botanical insecticides on percentage weight loss and protein content of treated black gram seeds exposed to *C. chinensis* attack after 6 months of storage

	Treatment	Concentration	6 MAS*	
			Weight loss (%)	Protein content (%)
T1	*Vitex negundo* (Begunia leaf powder)	10 g/kg seed	0.40 (0.95)	13.51
T2	*Lantana camera* (Naguari leaf powder)	10 g/kg seed	1.15 (1.28)	16.54
T3	*Murrya koenegii* (Curry leaf powder)	10 g/kg seed	0.55 (1.02)	15.75
T4	*Citronella mucronata* (Citronella oil)	10 ml/kg seed	0.44 (0.97)	14.28
T5	*Acorus calamus* (Sweet flag formulation)	10 ml/kg seed	0.35 (0.92)	13.25
T6	Untreated control	-	3.55 (2.01)	18.63
	SEM (±)		0.03	0.11
	CD (0.05)		0.8	0.33
	CV (%)		1.80	5.29

* Mean of 4 replications, MAS = Months after storage
Figures in parentheses are transformed values

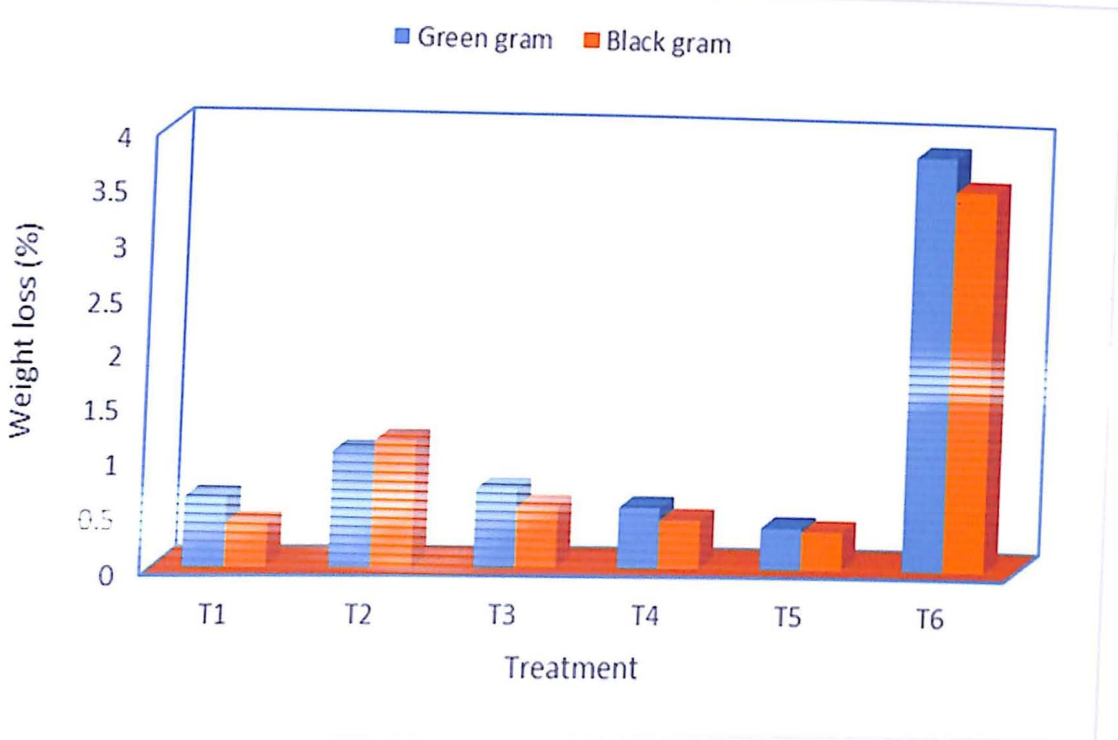


Fig. 16. Percentage weight loss of botanicals treated green gram and black gram seeds exposed to *Callosobruchus chinensis* L. after 6 months of storage

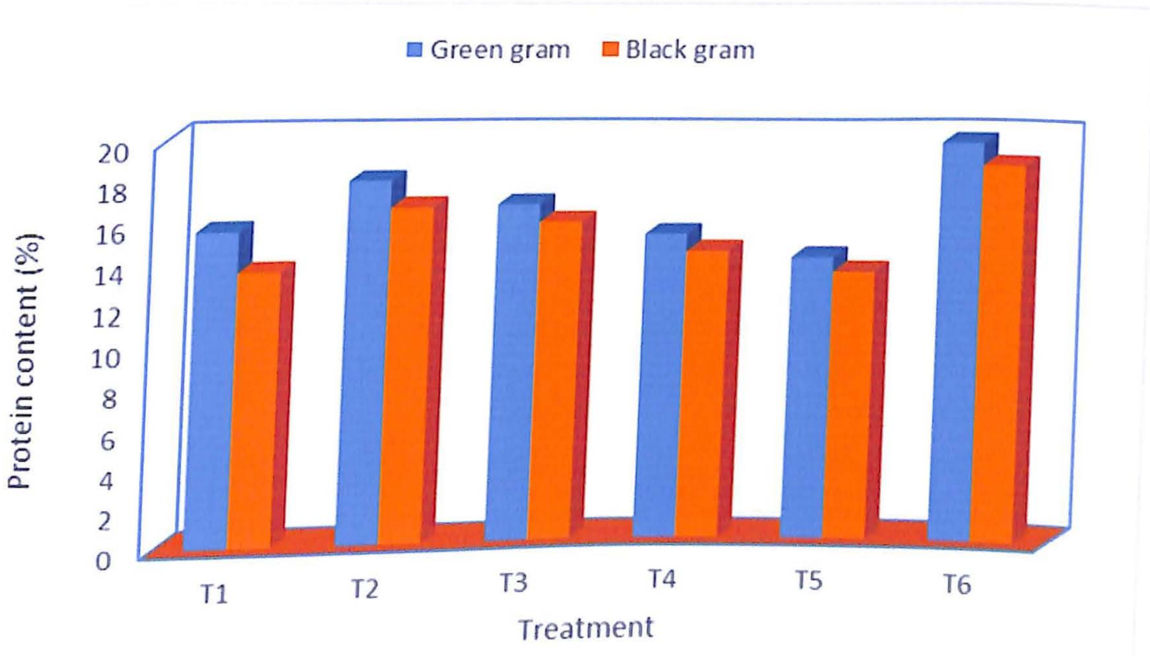


Fig. 17. Protein content of botanicals treated green gram and black gram seeds exposed to *Callosobruchus chinensis* L. after 6 months of storage

CHAPTER-V

DISCUSSION

DISCUSSION

Experiments were conducted in Seed Entomology Laboratory of Department of Seed Science and Technology, Orissa University of Agriculture and Technology, Bhubaneswar during 2014-15 to evaluate the performance of some insecticide molecules and botanicals against pulse beetle, *Callosobruchus chinensis* L. in treated green gram and black gram seeds under ambient condition during storage. The effect of insecticides and botanicals on mortality, biological parameters of pest and their impact on seed quality attributes were also studied. The quantitative losses and qualitative(biochemical) changes on protein composition of infested pulse seeds due to buchid infestation was also assessed towards later part of investigation after six months of seed treatment. The findings of laboratory experiments have been compared with available reviews of published literature and discussed below.

5.1 Adult mortality

5.1.1 Effect of insecticides on mortality of *Callasobruchus chinensis* in treated pulse seeds

Complete mortality of adult insects in all the treatments at 7 DAR (Days after release) except novaluron (95 %) was noticed in freshly treated green gram seeds (1 DAST). The incidence of mortality gradually declined in treated seeds with lapse of time during storage. After 6 months of storage period, the deltamethrin treatment recorded maximum mortality (77.50 %) in treated green gram seeds followed by emamectin benzoate (75.50 %), whereas in black gram it was highest in emamectin benzoate treatment (72.50 %) followed by spinosad (67.50 %) for the corresponding period. On the other hand, profenofos registered a maximum of 72.50 and 62.50 per cent mortality of bruchids in green gram and black gram seeds respectively. Among insecticides, novaluron was less effective and recorded 57.5 and 45.0 per cent killing of the pest in green gram and black gram seeds. It was observed that insecticidal effect against bruchid was comparatively more pronounced in green gram than black gram seeds. All the test insecticides proved more or less effective against the pest with higher residual effect except novaluron, being emamectin benzoate, spinosad and deltamethrin were superior to others.

The efficacy of synthetic pyrethroids i.e., (deltamethrin, cypermethrin, bifenthrin, fenvelarate and Cyfloxylate etc.) and organophosphates (Malathion, dichlorvus) at different doses causing higher mortality of adult bruchids in treated pulse seeds of moth bean, black gram and green gram up to 3-6 months storage period have been reported earlier by several workers (Patil *et al.*, 1994; Kalyan and Dadhich, 1999; Singh and Yadav, 2001; Prusty, 2002 and Pathania and Thakur, 2012). Further, Sanon and coworkers (2010) opined that by coating cowpea seeds with spinosad caused high mortality of adult *Callosobruchus maculatus* after 6 months of storage than deltamethrin in a laboratory experiment.

Multilocation Seed Entomology trials conducted at RAU (Durgapura), PDKV(Akola), MPKV (Rahuri) and TNAU (Coimbatore) centres indicated that higher mortality of bruchids was obtained with emamectin benzoate, spinosad, profenofos and deltamethrin treated green gram and black gram seeds up to 9 months after seed treatment in storage (Annual Report, AICRP on STR, NSP (Crops), 2013-14 and 2014-15). The present studies corroborate the observations of the above workers.

5.1.2 Effect of botanicals on mortality of *Callosobruchus chinensis* L. in treated pulse seeds

Sweet flag formulation caused highest mortality (97.5 %) followed by citronella oil (92.5 %) and begunia leaf powder (90.0%) at 7 DAR of pulse beetle in freshly treated green gram seeds (1 DAST). In black gram it was recorded maximum in sweet flag formulation treatment (100.0 %) followed by begunia leaf powder (95.0 %) and citronella oil (92.5 %). On 7 days exposure of pulse beetle after 6 months of seed treatment, sweet flag formulation also registered maximum of 67.5 and 75.0 per cent adult mortality followed by citronella oil (65.0 and 62.5 %) and begunia leaf powder (62.5 and 65.0 %) in green gram and black gram seeds, respectively. Naguari leaf powder was least effective and brought about only 45.0 per cent adult mortality after 6 months of seed treatment in both the pulse seeds. Among botanicals, sweet flag formulation consistently proved to be most effective in causing the higher mortality of bruchids followed by alternation in position between citronella oil and begunia leaf powder at different storage intervals. The effect of botanicals was more evidenced in black gram than green gram seeds against bruchids.

Efficacy of sweet flag rhizome powder @ 3-10 % and dried leaf powders of begunia @ 3 % in inducing higher mortality of adult bruchids as grain protectant in pulses has been studied previously by (Mishra, 2000 and Devi and Kalita, 2011).Govindon and Nelson (2007) observed cent per cent mortality of *Callosobruchus maculatus* adult in black gram treated with sweet flag rhizome powder @ 0.75-2.0 % at 48 hours after treatment. *Acorus calamus* 10 D @ 3 % caused 100 per cent mortality of bruchids after 72 hours and 84 hours of treatment when dust formulations were prepared using talc and fly ash as filler, respectively. Further Rahman and Co-workers (2006) have reported that begunia oil extract was more effective of 3 extracts tested i.e., (begunia (nishinda), eucalyptus and banklmi) and caused highest mortality of *Callosobruchus maculatus* in treated black gram seeds. Multilocational Seed Entomology trials conducted at ANGRU (Hyderabad), TNAU (Coimbatore), PDKV (Akola), JNU (Jamnagar), and CSAU&T (Kanpur) on various pulse seeds revealed that sweet flag formulation, begunia (nirgudi) and citronella oil were found promising and caused higher mortality of adult bruchids up to 6 months after treatment as compared to others. Thus, the present study is in agreement with the results of above scientists.

5.2 Oviposition

5.2.1 Effect of insecticides on oviposition of *Callosobruchus chinensis* L. in treated pulse seeds

It is evident from results that the bruchids laid minimum number of eggs on green gram seeds treated with emamectin benzoate (1.75 and 3.75) followed by deltamethrin (2.75 and 4.75 eggs) at 2 and 4 MAST, respectively. After 6 months of seed treatment, least number of eggs was visualized in deltamethrin (7.25 eggs) followed by emamectin benzoate (9.00 eggs). In black gram lowest oviposition was noted on emamectin benzoate (3.0, 6.5 and 8.75 eggs) followed by spinosad (3.25, 4.25, 9.75 eggs) after 2, 4 and 6 months storage period respectively. The untreated control showed maximum of 75.50 and 82.50 number of eggs in green gram and black gram, respectively when the seeds were exposed to bruchid attack after 6 months of treatment. It is clear from the findings that the fecundity decreased as the storage period increased. The emamectin benzoate, deltamethrin and spinosad had better antioviposition effect and suppressed fecundity of bruchids as compared to other treatments.

Santhi and Co-authors (1993) reported that the synthetic pyrethroids i.e., deltamethrin @ 20 ppm and cypermethrin @ 50 ppm supressed oviposition of *Callosobruchus maculatus* for only up to 2 months while at higher concentration @ 40 ppm and 100 ppm, the effect persisted up to 5 months as compared to higher fecundity in malathion. Effectiveness of synthetic pyrethroids i.e., deltamethrin and fenvalerate as seed protectant in reducing the egg laying capability of *Callosobruchus maculatus* on pigeon pea seeds up to 12 weeks period than other pyrethroids and malathion has been earlier studied by Patil and Co-scientists (1994). Sanon and Coworkers (2010) were of opinion that the biopesticide spinosad decreased the number of eggs laid by female *Callosobruchus macculatus* in cowpea seeds through out 6 months storage as compared to deltamethrin. In present findings both spinosad and deltamethrin minimized the oviposition by beetle in treated seeds which was in accordance with studies conducted by above authors.

5.2.2 Effect of botanicals on oviposition by *Callosobruchus chinensis* L. in treated pulse seeds.

It was noticed that least number of eggs were laid in sweet flag formulation (3.75-8.00 eggs) followed by citronella oil treatment (7.00-11.50 eggs) during the entire period of study in green gram seeds. Similarly bruchids oviposited lowest number of eggs in black gram seeds treated with sweet flag formulation (3.25-8.50 eggs) followed by begunia leaf powder (5.75 -10.75 eggs). Citronella oil and curry leaf powder showed a maximum of 11.50 to 12.50 eggs in green gram and 12.00 to 15.50 eggs in black gram after 6 months of seed treatment. Naguari leaf powder was comparatively inferior among botanicals and recorded 29.0 and 26.75 number of eggs at 6 MAST in green gram and black gram, respectively.

The similar findings on effect of plant products in reducing the oviposition by beetle have been published by following scientists. Rahman and Coworkers (2006) have stated that powdered leaves of begunia and eucalyptus and bankalmi(*Ipomea sepiaria* K.) @ 3 % mixture reduced oviposition of beetle in treated black gram seeds. Devi and Kalita (2011) evaluated some plant powders @ 3 g, 5 g and 10 g/per kg of green gram as post harvest grain protectants against *Callosobruchus chinensis* and observed sweet flag powder was the most effective in inhibiting the egg laying by bruchid closely followed by black pepper, neem, begunia and naguari leaf powder.

Decreased fecundity by bruchids infesting pulse seed grains treated with different vegetable oils (edible, non-edible and hair oils) has been studied by several past workers (Kachare *et al.*, 1994; Singh *et al.*, 1994; Khanna, 1995; Reddy *et al.* 1999; Mishra, 2000; Bhatanagar *et al.*, 2001 and Singh, 2003). These oils had adverse effect on oviposition preference and showed significant repellent, ovipositional deterrent effect for egg laying by bruchids up to 6 months and resulted in low fecundity in treated seeds. Chaubay (2013) conveyed about 2 essential oils of *Zingiber officinale and Piepr cubeba* that reduced oviposition potential of *Callosobruchus chinensis* adults when treated with sublethal concentration by fumigation and contact method. Oviposition inhibition was more pronounced when adults came in contact than in vapours). The citronella oil having strong pungent odour,repellent and oviposition deterrent action,fumigant and contact toxicity against eggs lowered the fecundity of female bruchids. Several plant formulations were tested and found promising against bruchids. Kirado and co-workers (2004) reported that ethanol extract, crude extract and powdered suspension of *Ocimum* sp. were noted to significantly bring down the number of eggs laid by beetle on mung bean than other formulation.The findings suggested that formulations of both species of *Ocimum* i.e., *basilum* and *sanctum* have a potential to act as ovipositional deterrent can be employed against *C chinensis* for its control. Mandal and Coworkers (2005) opined that neem in water, ethanol, methanol and acetone was most effective in terms of lower fecundity of *Callosobruchus chinensis* in bengal gram seeds followed by begunia and *Ipomea* spps. at 1, 3 and 6 hours after treatment, respectively. In present investigation, sweet flag (TNAU, formulation) had antioviposition effect that reduced the number of eggs on treated seeds The present findings commensurate the studies of above workers.

5.3 Population build up/Emergence of adult *C chinensis* L. (F_1)

5.3.1 Effect of insecticides on population build up of *Callosobruchus chinensis* in treated pulse seeds.

It was noticed that minimum number of adults emerged in emamectin benzoate (1.25-7.0 adults) followed by deltamethrin (2.50-8.25 adults) and spinosad treatment (2.75-8.75 adults) in green gram seeds throughout the period of investigation. The insecticidal effect on adult population build up was similar in blackgram seeds. Black gram seeds treated with emamectin benzoate sheltered (2.25

Decreased fecundity by bruchids infesting pulse seed grains treated with different vegetable oils (edible, non-edible and hair oils) has been studied by several past workers (Kachare *et al.*, 1994; Singh *et al.*, 1994; Khanna, 1995; Reddy *et al.* 1999; Mishra, 2000; Bhatanagar *et al.*, 2001 and Singh, 2003). These oils had adverse effect on oviposition preference and showed significant repellent, ovipositional deterrent effect for egg laying by bruchids up to 6 months and resulted in low fecundity in treated seeds. Chaubay (2013) conveyed about 2 essential oils of *Zingiber officinale and Piepr cubeba* that reduced oviposition potential of *Callosobruchus chinensis* adults when treated with sublethal concentration by fumigation and contact method. Oviposition inhibition was more pronounced when adults came in contact than in vapours). The citronella oil having strong pungent odour,repellent and oviposition deterrent action,fumigant and contact toxicity against eggs lowered the fecundity of female bruchids. Several plant formulations were tested and found promising against bruchids. Kirado and co-workers (2004) reported that ethanol extract, crude extract and powdered suspension of *Ocimum* sp. were noted to significantly bring down the number of eggs laid by beetle on mung bean than other formulation.The findings suggested that formulations of both species of *Ocimum* i.e., *basilum* and *sanctum* have a potential to act as ovipositional deterrent can be employed against *C chinensis* for its control. Mandal and Coworkers (2005) opined that neem in water, ethanol, methanol and acetone was most effective in terms of lower fecundity of *Callosobruchus chinensis* in bengal gram seeds followed by begunia and *Ipomea* spps. at 1, 3 and 6 hours after treatment, respectively. In present investigation, sweet flag (TNAU, formulation) had antioviposition effect that reduced the number of eggs on treated seeds The present findings commensurate the studies of above workers.

5.3 Population build up/Emergence of adult *C chinensis* L. (F_1)

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It was noticed that minimum number of adults emerged in emamectin benzoate (1.25-7.0 adults) followed by deltamethrin (2.50-8.25 adults) and spinosad treatment (2.75-8.75 adults) in green gram seeds throughout the period of investigation. The insecticidal effect on adult population build up was similar in blackgram seeds. Black gram seeds treated with emamectin benzoate sheltered (2.25

-8.25 number of adults) followed by deltamethrin (2.75-9.75 adults) and spinosad (3.50 -11.25 adults). Though all the insecticides were superior over untreated control, the above 3 insecticides afforded excellent protection in minimizing the population build up of adult bruchids. The insect growth regulator novaluron harboured maximum of 32.50 and 30.25 number of adults as against 75.50 and 84.50 number in untreated control in greengram and black gram, respectively after 6 months of seed treatment.

The efficacy of synthetic pyrethroids in suppressing the emergence of adult bruchids up to 3 to 5 weeks period after seed treatment has earlier been observed by Santhi *et al.* (1993) and Patil *et al.* (1994). According to Abo-elghar and Coworkers (2004), the chitin synthesis inhibitor (CSI) i.e., teflubenzuron reduced adult emergence of *Callosobruchus maculatus* (F_1 progeny) achieving control ranging from 96.2 % at 1 month to 94.30 % at 8 months which contradicted the present result. In present study the CSI novaluron was less effective and resulted higher population build up of bruchids. The authors also further observed strong reduction of adult emergence in organophosphate (OP) compounds i.e., pirimiphos methyl applied @ 25 mg/kg of cowpea seeds with 91.6 % control at 8 months post treatments which was in accordance with present study where OP compound profenofos checked the population build up of adult bruchids (F_1) more effectively. Sanon *et al.* (2010) have stated that the number of bruchids, *Callosobruchus maculatus* emerging from cowpea seeds was reduced by more than 80.0 % by coating the seed with spinosad but only by 43.0 % by coating with deltamethrin after six month of storage. In present work both the insecticides were effective and minimized adult emergence in treated seeds.

5.3.2 Effect of botanicals on population build up of *Callosobruchus chinensis* in treated pulse seeds

Among different botanicals tested minimum number of adults emerged in sweet flag formulation (2.75-7.25 adults) followed by citronella oil (5.00-11.00 adults) and begunia leaf powder (5.50 -14.50 adults) from green gram seeds during the period of study. In black gram, sweet flag formulation also showed minimum population build up (1.75-6.25 adults) followed by begunia (4.75-12.50 adults) and citronella oil (5.75-14.50 adults). These three plant products were more effective in preventing embryonic development transformation to F_1 adult bruchids. Naguari leaf powder was comparatively less effective and harboured a maximum of 30.00 and 25.75 adults as compared to 85.25 and 80.75 adults in untreated control after 6 months of treatment in green gram and black gram seeds, respectively.

Similar findings were reported by Devi and Kalita (2011) who reported that sweet flag powder @ 3, 5 and 10 g/kg of green gram as grain protectant against *Callosobruchus chinensis* recorded minimum adult emergence (F_1) followed by black pepper. Efficacy of different plant materials as seed protectant (turmeric rhizome powder, neem leaf powder, black pepper seed powder and clove powder etc.) in reducing the emergence of adult bruchids (F_1 population) on pulse seeds up to 4 months of storage had been studied previously by Gill and Singh (2012), Bhubaneswari Devi and Vectoria Devi (2013), Neog and Singh (2013) and Patro *et al.* (2014). Several past workers have opined that oils of neem, karanj, castor, mustard, soybean, sesame, groundnut, coconut, pamolin, mahua etc at various concentrations as surface protectants caused significant reduction in emergence of adult *Callosobruchus chinensis* on different pulse grains up to 3 to 9 months after seed treatment in storage (Lakhan Pal *et al.*, 1995; Reddy *et al.*, 1999; Singh, 2003; Raman *et al.*, 2006 and Srieekanth *et al.*, 2011) In present endeavour the the ability of sweet flag formulation and begunia leaf powder to effectively reduce the expected emergence as compared to others is an indication of presence of toxic components in them. The mixing of citronella oil smoothened the seed surface so that beetle could not proliferate on smooth seed coat. Thus the present study is in conformity with the findings of above authors.

5.4 Seed damage

5.4.1 Impact of insecticides on seed damage by *Callosobruchus chinensis L.*

In green gram lowest seed damage was recorded in emamectin benzoate (1.00 %) followed by deltamethrin and spinosad (1.25 %) after 6 months storage period. On the other hand the organophosphate insecticide profenofos andinsect growth regulator novaluron registered 2.50 and 3.25 % seed damage respectively as against maximum damage in untreated control (10.75 %). The seed damage was found minimum in spinosad (1.50 %) followed by emamectin benzoate and deltamethrin treatment (2.00 %) after 6 months of storage in treated black gram seeds. In black gram, profenofos and novaluron treatment exhibited a maximum of 3.75 and

4.50 % seed damage after 6 months of seed treatment in storage as compared to 12.50 % in control. The percentage reduction in seed damage among insecticidal treatments over control ranged between 69.77 to 90.70 in green gram whereas in black gram the corresponding reduction varied from 64.0 to 88.0 % after 6 months of storage.

Sanons and Coworkers (2010) determined the efficacy of spinosad and synthetic pyrethroid deltamethrin against cowpea weevil, *Callosobruchus maculatus* F. and reported that 20.0 % seeds were perforated in the spinosad treatment compared with 29.0 % for deltamethrin. Spinosad controlled the pest through out the 6 months cowpea storage which was in agreement with present study. Rajjsri *et al.* (2012) recorded highest seed damage of bengal gram in deltamethrin treatment indicating development of resistance in *Callosobruchus chinensis* against commonly used synthetic pyrethroid insecticide deltamethrin which contradicted the present study. Multilocational Seed Entomology trials conducted at different centres during 2013-14 and 2014-15 indicated pulse seed treated with new molecules i.e., emamectin benzoate, spinosad, rynaxypyr, chlorfenapyr, profenofos along with deltamethrin witnessed significantly lower seed damage than that of untreated control after six months of seed treatment which corroborates the present findings(Annual Report. AICRP on STR, 2013-14 and 2014-15).

5.4.2 Effect of botanicals on seed damage by *Callosobruchus chinensis L.*

Among botanicals, sweet flag formulation controlled the pest most effectively and showed least infestation (1.25-2.25 %) in treated green gram seeds followed by citronella oil (1.25-2.50 %) and begunia leaf powder (1.50-2.50 %) throughout entire period of investigation. Seed treatment with curry and naguari leaf powder contributed moderate seed damage of 4.25 and 5.25 % respectively as compared to 13.00 % in control. Botanical treatments in green gram seeds contributed a reduction of 59.61 % to 82.69 % over control after 6 months of storage period. In black gram, begunia leaf powder gave maximum protection (1.50 %) followed by sweet flag formulation (1.75 %) and citronella oil (2.25 %) after 6 months of storage. A reduction advantage of 67.39 to 86.96 % in seed damage over control was estimated by different botanical treatments after 6 months of storage in black gram seeds. All the botanicals provided better protection of black gram against bruchids than that of green gram seeds.

Effectiveness of botanicals i.e., dried powder of neem cake, dried neem leaf powder, neem bark powder, turmeric rhizome powder and clove powder at different concentrations for grain protectant against bruchid in different pulses has been documented by several past researchers (Singh and Pandey, 1995; Singh, 2003; Gill and Singh, 2012 and Patro *et al.*, 2014).Devi and Kalita during 2011 evaluated various plant powders at different doses @ 3 to 10 g/kg as seed protectant against *Callosobruchus chinensis* and found that sweet flag powder lowered the incidence of seed damage followed by black pepper in stored green gram up to 6 months after treatment. In present work sweet flag formulation most effectively controlled seed damage. Rahman and co-authors (2006) viewed that powdered leaves and extracts of nisinda (begunia), eucalyptus and bankalmi at a 3 % mixture decreased the seed infestation considerably and provided excellent protection against *Callosobruchus maculatus* in blackgram.

Pulse seeds smeared with different types of plant oils @ 8 to 15 ml/kg of seeds minimized the seed infestation up to 4-9 months storage period were reported by Khanna (1995), Singh *et al.* (2001), Singh, 2003 and Jangamashetti *et al.* (2008). Multilocational Seed Entomology trials conducted by Entomologists at different centres i.e., TNAU (Coimbatore), CSAUA&T (Kanpur), PDKV (Akola), JAU (Jamnagar), UAS (Bangalore) and NDUA&T (Faizabad) revealed least seed infestation by bruchids up to 6 months storage period when the pulse seeds were treated with sweet flag formulation, begunia leaf powder and citronella oil than other botanicals which supplements the present investigation (Annual Report, AICRP on STR, 2014-15).

5.5 Storability of treated pulse seeds against *C chinensis* L.

5.5.1 Effect of insecticides on seed quality parameters against *Callosobruchus chinensis* L.

After 6 months of seed treatment, emamectin benzoate expressed higher germination and vigour index (86.75 % and 2094) followed by deltamethrin (85.00 % and 2122) and spinosad treatment (83.50 % and 1948) in green gram. The moisture content ranged between 10.0 % (emamectin benzoate) to 11.6 % (untreated control) among different treatments after 6 months storage in treated seeds. In black gram seeds maximum germination count obtained in emamectin benzoate (85.5 %)

followed by spinosad (83.75 %) and deltamethrin (81.75 %) after 6 months of seed treatment. Similar trend was noticed for vigour index (VI) which was highest in emamectin benzoate (2020) followed by 1974.0 and 1865.7 in spinosad and deltamethrin treatments, respectively. The moisture percentage varied from 10.1 (emamectin benzoate) to 11.9 (control) in blackgram seeds. It is clear from result that although the seed germination count and VI gradually decreased due to increased seed infestation by the pest with lapse of time and loss of residual effect of insecticides during storage,still both the pulse seeds maintained germination potentiality above IMSCS i.e., more than 75 % after six months storage.

Patil and Coworkers (1994) studied efficacy of synthetic pyrethroids along with malathion against *C. maculatus* on pegionpea seeds and stated that none of insecticidal treatment affected seed germination adversely up to 12 weeks period in storage. Further, Gupta and Coscientists (1998) reported that seed treatment with deltamethrin 0.04 ml/kg and thiram @ 2.5 g/kg against *C. chinensis* did not hamper the viability of mungbean seeds in gunny bagsfor 18 months. Further Rajjsri and Co-authors (2012) observed negative impact of seed treatment with deltamethrin for the control of *C. chinensis* that resulted in poor germination and vigour of stored bengal gram.

Results of Multilocational Seed Entomology trials conducted at various centres indicated that different pulse seeds treated with new molecules i.e., emamectin benzoate, rynaxypyr, chlorfenapyr, spinosad, profenofos along with deltamethrin against pulse beetle recorded significantly higher germination and vigour index potential of pulse seeds up to 6-9 months of storage as compared to control with no adverse effect (Annual Report, AICRP on STR, 2013-14 and 2014-15).Hence the present findings are in good agreement with earlier reports of above scientists.

5.5.2 Effect of botanicals on seed quality attributes against *C. maculatus* L.

In green gram seeds, sweet flag formulation expressed maximum germination count (82.0 %) followed by citronella oil (81.0 %) and begunia and curry leaf powder (78.0 %) as compared to 67.0 % in untreated control after six months of seed treatment. Sweet flag formulation also exhibited highest vigour index (2081) followed by begunia leaf powder (1953) and citronella oil (1910). The moisture content was least in sweet flag formulation (10.4) as against maximum in control treatment (12.4

%). In black gram seed, maximum germination count and vigour index was recorded in sweet flag formulation (85.0 % and 2072) followed by begunia leaf powder (84.0% and 1995) after 6 months of seed treatment during storage. The germination and vigour index in citronella oil (82.0% and 1852) and curry leaf powder (82.0 % and 1820) treatments differed distinctly from that of naguari leaf powder (75.0 % and 1712) and untreated control (71.0 % and 1419). The moisture content of black gram seeds was minimum in begunia leaf powder and sweet flag formulation treatment (10.2 %) as compared to (12.2 %) in untreated control.

None of the botanicals (plant powders and extracts) used as seed protectant against bruchids, *C chinensis* L. adversely affected the germination of pulse seeds up to 4-6 months storage period as reported by Yadav and Bhargav (2005), Gill and Singh (2012), Singh *et al.* (2012) and Neog and Singh (2013).Sanon and Coworkers (2010) opined that sweet flag rhizome powder treated with cowpea seeds maintained the seed viability at appreciable level up to 6 months storage after seed treatment which was in accordance with present findings.

Mixing of different plant products and vegetable oils in Rajma beans against *C. chinensis* did not affect the germinability of seeds at 3 and 180 days after treatment. Among all treatments oils proved better, especially coconut oil followed by niger and sesame oil have been reported by Sreekanth and Coworkers (2011). Mixing of plant oils with pulses did not show adverse effect on seed germination after 3 to 6 months of seed treatment has been studied previously by (Rahman et al.,2006 and Chaubey *et al.*,2013). In present investigation citronella oil recorded good germination and vigour index of both the treated pulse seeds and did not show any adverse effect on seed germination.

5.6 Quantitative and qualitative(biochemical) changes

5.6.1 Effect of insecticides on percentage weight loss and protein content in treated pulse seeds due to *C chinensis* invasion

The percentage weight loss was minimum in emamectin benzoate (0.22 %) followed by deltamethrin (0.24 %) and spinosad (0.28 %) as compared to control treatment (2.82 %) after 6 months of storage of treated green gram seeds.In black gram, lowest seed weight loss was visualized in emamectin benzoate (0.33 %) followed by spinosad (0.39 %) and deltamethrin (0.46 %) whereas highest weight loss was estimated in untreated control (4.07 %). Increased seed infestation (%) recorded higher percentage of weight loss of seed and vice-versa. The percentage reduction in weight loss over untreated control between 9.47 to 29.32 and 11.22 to 28.87 was estimated among different insecticidal treatment of green gram and black gram seeds, respectively.

Patil *et al.* (1994) observed that pegionpea seeds treated with synthetic pyrethroids i.e., deltamethrin (10, 12.5 ppm), fenvalarate (60, 75 ppm) and cyfloxylate (150 ppm) recorded no loss in seed weight up to 12 weeks period due to complete protection against *Callosobruchus maculatus* whereas treatment with malathion (50 ppm) exhibited higher loss in grain weight at 4 weeks after treatment. Studies against pulse beetle in stored black gram by using insecticides indicated that malathion @ (0.5 %) was found best in minimizing weight loss of seeds (0.28%) than other insecticides due to least seed damage as reported by Pathania and Thakur (2012). The findings of present study is supported by the studies conducted by above researchers.

The estimated protein content of infested green gram seeds after 6 months storage indicated lowest protein value in emamectin benzoate(13.44) followed by deltamethrin (13.75) as compared to highest protein composition in untreated control (18.44 %).In black gram seed protein value was analysed significantly minimum in emamectin benzoate (12.96 %) followed by spinosad (13.90%) as against maximum in untreated control (19.84%). It was found that the treatments which recorded higher seed damage by bruchids, had higher protein content and vice-versa. Infestation by bruchids significantly increased the protein composition of treated seeds.

Umrao and Verma (2003) observed that the pea varieties having low protein content was least susceptible while varieties with higher protein content were highly susceptible to pulse beetle, *C. chinensis* L. The protein content of each variety significantly increased due to bruchid infestation after 120 days irrespective of susceptibility parameters of varieties. Further, Neog and Singh (2011) opined that the total soluble sugar, non-reducing sugar, starch and crude fat content decreased while moisture, reducing sugar, crude protein, free fatty acid and uric acid content increased significantly in different pulse seeds due to infestation by *C. chinensis* L. after 60 and 90 days of storage as compared to healthy ones which was in accordance with study of Umrao and Verma.Thus the present findings is in agreement with findings of above scientists where increased protein composition was recorded due to bruchid infestation. In present finding the protein composition in different treatments increased significantly due to bruchid infestation in treated seeds which commensurate with the findings of Umrao and Verma (2003) and Neog and Singh (2011).

Pal and Co-authors (2011) in a study concluded that green gram varieties with low protein and higher oil content were least susceptible whereas those with high protein and low oil content were highly susceptible. Higher protein content in fresh seed of the varieties led to more infestation. There was considerable reduction in protein content after *C. chinensis* feeding which was not in conformity with the present investigation. Singh *et al.* (2004) reported that pulse varieites with higher growth index value (higher susceptibility) had the lower protein content i.e., susceptibility to C. chinensis of a pulse variety is negatively correlated with its protein content which was contradictory to opinion of earlier author Umrao and Verma. .

5.6.2 The effect of botanicals on percentage weight loss and protein content of treated pulse seeds due to *C chinensis* invasion

The estimated weight loss in various treatments ranged between 0.38 % (sweet flag formulation) to 3.86 % (control) and 0.35 % (sweet flag formulation) to 3.35 % (control) in green gram and black gram seeds, respectively after six months storage period. The citronella oil and begunia leaf powder treated seed also showed a lower value of weight loss 0.55 and 0.63 % in green gram and 0.44 and 0.40 % in black gram seeds. On the contrary, significantly highest seed weight loss of 3.86 % and 3.55 % in untreated control was calculated in green gram and black gram seeds respectively due to maximum seed damage by bruchids. Botanical treatments recorded a reduction of 9.47 to 29.32 % and 11.22 to 28.88 % in seed weight loss over untreated control in green gram and black gram respectively.

The percentage seed weight loss was lower in pulse seeds treated with different botanicals as compared to untreated control due to low seed infestation by bruchids has been studied by many past researchers (Govindan *et al.*, 2009; Gill and Singh, 2012 and Singh *et al.*, 2012). Highest weight loss (47.40 %) was recorded in untreated pea grains which considerably reduced to a level of 0.63, 1.00 and 1.10 % by mixing seed with neem oil neem leaf powder and castor oil, respectively was reported by Singh and Coworkers (2001). Devi and Kalita (2011) have stated that the percentage weight loss was minimum in green gram treated with sweet flag powder followed by black pepper treatment due to low intensity of seed damage by bruchids. Sreekanth and Coworkers (2011) opined that coconut oil treated with rajma beans @ 1.5 ml/100 g seeds showed no weight loss up to 180 days after treatment due to nil infestation by bruchid. More than 30 % reduction in seed weight loss over control in various botanicals seed treatments against pulse beetle infestation was observed by Wast and Coauthors (2015). These reports are in corroboration with present findings.

The protein composition in different botanical treatments varied significantly from 13.96 % (sweet flag formulation) to 19.75 % (control) and 13.25 % (sweet flag formulation) to 18.63 % (control) per cent in green gram and black gram infested seeds, respectively after 6 months storage. The citronella oil and begunia leaf powder recorded a moderate protein value of 15.12 and 15.48 % in green gram and 13.25 and 13.51 % in black gram seeds. It was observed that the protein value was estimated more in seeds that suffered higher infestation due to low toxicity of botanicals against bruchids. These results gain support from Umrao and Verma (2003) and Neog and Singh(2011) who reported increase in protein value due to increased damage by bruchids. and in all the treatment the protein value increase significantly than that of previously taken before exposed to pulse beetle which was common in all treatments.

A highly positive relationship was found between the level of insect infestation and per cent increase of protein content in different treatments. Hence, it was evident that the insect infestation had a direct relation to the increase of crude protein content. This increase might be due to increase in uric acid, non-protein nitrogen and presence of insect body fragments and body parts inside the seeds. It is worthnoting that the uric acid is one of the end products of protein metabolism in insects. The insect population was higher at higher level of infestation which increased the uric acid and nonprotein nitrogen of pulses.

In the past, lot of studies have been conducted on effect of seed treating chemical insecticides discriminatingly of synthetic pyrethroids and few organo phosphates in pulses against *Callosobruchus* spp. The current piece of investigation on efficacy of insecticide molecules and effect of botanicals particularly citronella oil as seed protectant on pulses against bruchids, *Callosobruchus chinensis* L. is a new aspect of study. The published literature is not available to support the present findings. Now Entomologists of AICRP on STR have been carrying out experiments on performance of new molecules against bruchids spreading at different locations in the country. Hence, further effort needs to be oriented to concentrate research work on new boipesticides as their seed treatment against *Callosobruchus spp.* for safe storage of pulse seeds up to a desirable period to find out suitable and proper cost effective management strategy.

CHAPTER-VI

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Investigations were carried out in the Seed Entomology Laboratory of Department of Seed Science and Technology during 2014-15 to evaluate the bio-efficacy of new-insecticide molecules and botanicals as seed protectant against pulse beetle, *Collosobruchus chinensis* L. infestation for safe storage of green gram and black gram seeds under ambient condition. The insecticidal and botanical experiments were laid out in complete Randomized Design (CRD) with 6 treatments and replicated 4 times. The effect of insecticides and botanicals on mortality, biological parameters of pest and its impact on seed quality attributes was determined at bi-monthly intervals after seed treatment. Besides the quantitative and qualitative losses/changes due to bruchid infestation were also assessed after six months storage period in treated pulse seeds.The findings of the studies are summarized below.

It is evident from the results that different insecticides had significant variations in their toxicity in bringing down the mortality of adult bruchids, *C Chinensis* L. in treated pulse seeds. All the insecticide molecules caused cent per cent mortality of adult beetles in both the pulses except novaluron (95.0 %) in black gram on 7 days exposure to freshly treated seeds (1 DAST). The percentage of adult mortality decreased with increase in duration of treatment. At 6 months after seed treatment (MAST), maximum adult mortality of 77.50 and 72.50 per cent was recorded with the treatment of deltamethrin and emamectin benzoate in green gram and black gram seeds, respectively. The spinosad treatment caused 72.50 and 67.50 per cent adult mortality after six months of storage in treated seeds of green gram and black gram. The mortality percentage in deltamethrin (77.5), emamectin benzoate (75.5), spinosad (72.5) and profenofos (72.5) seed treatments were at par with each other in green gram. In black gram, highest mortality recorded in emamectin benzoate (72.50 %) was equally effective with that of spinosad (67.50) but significantly superior to other treatments. The treatment with insect growth regulator novaluron was found least effective and resulted 57.5 % cumulative mortality of adult beetles in green gram and 45.0 % in black gram.

Significant variations in mortality count of pulse beetles were found when the seeds were treated with different botanicals. All the treatments showed superiority over control in killing the pulse beetles. The treatment with sweet flag formulation resulted in highest cumulative mortality of adult bruchids (97.5 %) following 7 days exposure to treated seeds after 6 months of storage. In black gram, cent per cent cumulative mortality was noticed in sweet flag formulation followed by begunia leaf powder treatment (95.0 %) after 7 days when bruchids were released at 1 day after seed treatment (DAST). Following 7 days of exposure after 6 months of seed treatment, the mortality percentage in treatments of sweet flag formulation (67.5 %), citronella oil (65.0 %) and begunia leaf powder (62.5 %) were comparable to each other in green gram. In black gram, maximum mortality (75.0 %) was witnessed in seed treated with sweet flag formulation differed significantly from others. The mortality recorded in begunia leaf powder (65.0%) was at par with citronella oil (62.5 %). The seeds mixed with curry leaf powder showed a maximum of 55.0 and 57.5 % mortality after 6 months of treatment in green gram and black gram, respectively whereas no mortality was observed in untreated control.

While observing the efficacy of insecticides on oviposition it was found that minimum number of eggs (7.25) recorded in green gram seeds treated with deltamethrin was at par with emamectin benzoate (9.00 eggs) after 6 months of seed treatment. In black gram, minimum oviposition was noted in emamectin benzoate (8.75 eggs), which was statistically at par with spinosad (9.75 eggs) and deltamethrin (11.25 eggs). Among insecticidal treatments, highest oviposition was noticed in case of novaluron (26.00 and 21.75 egg) in green gram and black gram, respectively. All the insecticides proved superior to control in reducing oviposition where maximum number of eggs (75.50 and 82.50) were laid on green gram and black gram seeds after six month of seed treatment.

Among botanicals fecundity was significantly minimum in sweet flag formulation (8.0 eggs) followed by citronella oil (11.50 eggs) and begunia leaf powder (17.0 eggs) after six month of treatment in green gram. In black gram, lowest oviposition visualized in sweet flag formulation treated seeds (8.5 eggs) were statistically on par with begunia leaf powder (10.75 eggs) and superior over rest of the treatments (12.00-26.75 eggs). The untreated control recorded a maximum of 90.50 and 82.50 eggs in green gram and black gram seeds after six months of seed treatment.

The population build up of adult bruchids varied significantly from 7.00 to 75.50 and 8.25 to 84.50 among insecticides including untreated control after six months of seed treatment in green gram and black gram, respectively. The minimum number of adults emerged from seed treatment with emamectin benzoate (7.0) were found at par with that of deltamethrin (8.25) and spinosad (8.75) in green gram. In black gram, the population of adult bruchids emerged from emamectin benzoate (8.25) and deltamethrin (9.75) were comparable with each other and varied significantly from spinosad (11.25), profenofos (17.0) and novaluron (30.25) treated seeds. The insecticidal seed treatments showed a population reduction ranging between 56.95 to 90.73 per cent and 64.20 to 90.24 per cent over untreated control in green gram and black gram seeds, respectively.

Adult emergence in different botanical treatments was discernible both in green gram and black gram seeds. The green gram seeds treated with sweet flag formulation recorded lowest adult emergence (2.75-7.25 adults) throughout the period of investigation followed by citronella oil (5.00 -11.00 adults) and begunia leaf powder (5.50-14.50 adults). In black gram, sweet flag formulation harboured less number of F_1 adults (1.75-6.25 adults) as compared to begunia leaf powder (4.75 - 12.50 adults) and citronella oil (5.75-14.50 adults). On the contrary, the seed treatment with curry leaf powder produced a maximum population of 18.50 and 19.25 number of adults as against 85.25 and 80.75 number adults emergence in untreated control after six month storage in treated green gram and black gram seeds.The seed treatment with naguari leaf powder was not so much effective in inhibiting the progeny development. A population reduction of 64.81 to 91.49 % and 68.11 to 92.26 % over control was estimated among botanical treatments after six months of storage in green gram and black gram seeds, respectively.

After 6 months of seed treatment emamectin benzoate was most effective with 1.0 % seed infestation in green gram and remained at par with spinosad and deltamethrin (1.25 % seed damage). On the other hand, bruchids damaged 2.50 and 3.25 % green gram seeds in profenofos and novaluron treatments as compared to 10.75 % in control. In black gram, minimum seed damage was recorded in spinosad (1.50 %) and it did not show significant variation from that of deltamethrin and emamectin benzoate (2.00 %).The profenofos and novaluron were moderately effective against the pest and registered 3.75 and 4.50 per cent seed damage, respectively as against 12.50 per cent in control. Highest reduction of seed damage over control after 6 months of storage period was calculated in emamectin benzoate (90.70) and spinosad (88.0) in green gram and black gram seeds, respectively.

The effect of botanicals on reduction of seed infestation of green gram and black gram by bruchids is evident from the present studies. The effectiveness of botanicals reduced with increase in storage period. After six months of storage,the infestation in treated seeds varied from 2.25 to 5.25 % and 1.50 to 3.75 % as against 13.00 and 11.50 % in untreated seeds of green gram and black gram, respectively. The per cent reduction of seed infestation over control after six month of storage was recorded highest in sweet flag formulation (82.69) in green gram and begunia leaf powder (86.96) in black gram.

The insecticidal effect on viability of green gram and black gram seeds revealed that none of the insecticides adversely affected the seed germinability up to six months of storage period. The germination potential of seeds gradually declined with increase in storage periods. After 6 months of seed treatment emamectin benzoate expressed highest germination count (86.75 %) remained at par with deltamethrin (85.0 %) followed by spinosad (83.50 %) in green gram. In black gram, maximum germination count recorded in emamectin benzoate (85.50 %) was comparable with spinosad (83.75 %) followed by deltamethrin (81.75 %). Similar trend in vigour index (VI) was also noticed. The vigour index was highest in deltamethrin (2122) followed by emamectin benzoate (2094) and spinosad (1948) in green gram whereas in black gram maximum vigour index was recorded in emamectin benzoate (2020) followed by spinosad (1974) and deltamethrin (1865) at 6 months after seed treatment.. There was 6.85 to 18.83 and 6.90 to 17.93 per cent increase in germination count over control due to insecticidal seed treatments after six months storage period in green gram and black gram, respectively.

The germination percentage among botanical treatments varied significantly between 86.0 to 90.0 % after 2 months storage which declined after 6 months storage period and ranged between 72.0 to 82.0 % in treated green gram seeds. After 6 months of seed treatment, all the treatments except naguari leaf powder (72.0 %) showed good germination pergentage of green gram as compared 67.0 % in control.

Seed treatment with sweet flag formulation expressed highest germination and vigour index potential of green gram seeds (82.0 % and 2081) followed by citronella oil (81.0 % and 1910) and begunia leaf powder (78.0 % and 1953). After 6 months of seed treatment, all the botanicals registered germination count of black gram seeds above IMSCS i.e., more than 75 %. The germination percentage and vigour index was maximum in sweet flag formulation (85.0 % and 2072) followed by begunia leaf powder (84.0 % and 1995) and citronella oil (82.0 % and 1852). Seed treatment with sweet flag formulation after six months storage contributed a maximum of 22.39 and 19.72 per cent increase in germination percentage over control in green gram and black gram seeds.

The study on effect of insect infestation on changes of moisture content in the insecticidal treated seeds of green gram and black gram revealed lowest increase in emamectin benzoate (10.0-10.1 %) as compared to 11.0 % in novaluron treatment after six months of storage. Significantly highest increase (11.6-11.9 %) was observed in untreated seeds of both pulses.

Among botanicals increase in moisture content was minimum in sweet flag formulation (10.2 to 10.4 %) as against maximum increase in untreated control (12.2 to 12.4 %) after six months of storage in treated pulse seeds. Thus it was clear that moisture content in the seeds increased with increase in damage by bruchids.

Among insecticidal treatments minimum weight loss due to bruchid nfestation estimated in emamectin benzoate (0.22 %) was at par with deltamethrin (0.24 %) and spinosad (0.28 %) differed significantly from rest others (0.37-0.75 %) as compared to maximum control (2.82 %) after six months of storage in treated green gram seeds. In black gram emamectin benzoate also showed lowest weight loss (0.33 %) remained at par with spinosad (0.39 %) followed by deltamethrin (0.46 %) treatment.

The seed treatment with sweet flag formulation recorded maximum reduction in weight loss (90.15 %) over control followed by citronella oil (85.75 %) in green gram. In black gram botanical treamtents brought about a reduction of 67.60 (naguari leaf powder) to 90.14 per cent (sweet flag formulation) in seed weight loss over control.

The estimated protein content of infested green gram seeds after six months storage period indicated lowest protein value in emamectin benzoate (13.44 %) followed by deltamethrin (13.75 %) as compared to highest protein composition in untreated control (18.44%). In black gram seeds the protein value was analysed significantly minimum in emamectin benzoate 12.96) followed by spinosad (13.90) as against maximum in control (19.84 %).

Among botanical treatments the minimum increase in protein content was calculated in sweet flag formulation (13.96 and 13.25 %) in green gram (13.96 %) and black gram (13.25%) seeds due to low seed infestation by bruchids after 6 months of storage . The untreated control recorded maximum increase in protein value i.e.,19.75 % in green gram and 18.63 % in black gram due to highest seed damage.It is pertinent to mention that increased infestation by bruchids resulted in higher weight loss and rise in seed protein composition.

Based on present findings it can be concluded that there is great scope for using new molecules and locally available plant products as prophylactic seed treatment against cross infestation by *Callosobruchus* spp. for protection of stored pulse seeds at least 4 to 6 months period appreciably with no adverse effect on viability of seeds.

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