EVALUATION OF INSECTICIDAL ACTIVITY OF PONGAMIA PINNATA SEED EXTRACT AGAINST PULSE BEETLE, *CALLOSOBRUCHUS CHINENSIS* L

DR. GUNMALA GUGALIA ,

*Corresponding author

Associate Professor,

Department of Botany

Sangam University, Bhilwara(Raj.)

Dr. Nitin Kumar,**

Assistant Professor of Agriculture

Sangam University, Bhilwara

Ajay Kumar Jayswal***

Assistant Professor of Agriculture

Sangam University, Bhilwara

Abstract

Objectives: This study was undertaken the *Pongamia pinnata* Linn. seed extract as insecticide for integrated pest management against pulse beetle, *callosobruchus chinensis*

Methods: Petroleum ether extract, methanolic extract and aqueous extracts of *P. pinnata* seeds were used to coat the seeds of green gram at the rate of 1, 2 and 5 per cent concentrations. Five pairs of the newly emerged adults of *C. chinensis* were released in each plastic container which was covered with muslin cloth and was tightened with rubber band. The treatments, including control, were maintained in three replications. All the adults were allowed to remain in the container till their natural mortality under room temperature. The mortality of adult beetles released on treated seeds was recorded at 1, 3, 7, 10 and 15 days after treatment till complete kill, even in the control. The egg laying was recorded after 7 and 15 days of release of beetles in each treatment. The adult emergence was monitored at monthly interval for 2 months**.**

Results:

Various extracts *of Pongamia pinnata* seed such as petroleum ether , methanolic and aqueous extract were evaluated for protection of green gram seed from pulse beetle, *Callosobruchus chinensis L.* One day after treatment, maximum mortality (20.22%) was observed in petroleum ether extract coated seed was compared to methanol extract (16.7%). On day-3, petroleum ether coated seeds resulted 50.56% mortality and its 2 and 1 per cent concentrations were equally effective (50.00 and 43.33% mortality). On day-7, highest mortality (85.00%) was observed in petroleum ether coated seeds followed by methanol and aqueous extract (77.78%), (66.67%) . Complete mortality was recorded in petroleum ether extract coated seeds at 5 per cent concentration and was significantly higher than 2 per cent (90.00%) and 1 per cent (80.00%) concentration. After 15-day observation, Petroleum ether and methanol extract resulted 100.00 and 95.56 per cent kill, respectively. Maximum mortality was observed in seeds coated with petroleum ether (70.22%), followed by methanol extract (60.56%)and aqueous Extract (53.30%). The minimum number of eggs (4.70 eggs/5 females) recorded in petroleum ether coated seeds was statistically at par with methanol (6.60eggs/5 females). Minimum adult emergence (0.50 beetles) was recorded in petroleum ether coated seeds was statistically at par with methanol extract (0.85 beetles) after sixty day **.**

Conclusion: Petroleum ether extract of *P. pinnata* seed have great potential for sustainable integrated pest management programmes**..**

Key words*:* *Callosobruchus chinensis*, *P. pinnata*, Petroleum ether extract

INTRODUCTION

A number of insect-pests attacks the stored grains, seeds and other products. *Callosobruchus chinensis* L . (Bruchidae: Coleoptera) is the pulse beetle which is the important insect pests of stored grain, causes extensive losses to the pulses in the storage [1, 2] though the primary invasion occurs in the field itself. Commercial value of the seed is reduced due to weight loss, decreased germination potential of the seed [3,4]. It is a serious pest of pulses and has also been reported attacking cotton seed, sorghum and maize [5]. Farmers mainly rely on the use of pesticides for the control of these beetles. The random use of synthetic organic pesticides, however, has created a number of problems including insect resistance, toxic residues in food grains [6, 7]. The traditional farmers have poor storage structures in the developing countries are unsuitable for effective conventional chemical control, as most of the storage structures are open to re-infestation by insect-pests [8]. Methyl bromide fumigation has been the most widely applied management practice for the control of insect-pests, including *C. chinensis* in stored grain products [9].

However, the ozone depleting effect of methyl bromide has led to restrictions on its use, and the Montreal Protocol of the United Nations Environment Programme (UNEP) recommended the phasing out of methyl bromide by 2005 in developed and by 2015 in developing countries [10]. Insect are resistant against phosphine is becoming more common [11, 12] and is a matter of considerable concern. These chemical insecticides enter in the food chain and pollute the environment. Therefore, there is a need to develop safe alternatives of predictable insecticides and fumigants to protect stored seed grains from insect-pest invasions. The plant products were widely used as grain protectants is an age old practice [13] and these is quite safe and promising [14, 15]. Many botanicals have been reported to protect seeds from bruchids infestation in many pulse crops [16,17.18]. Therefore, there is a need to develop safe alternatives insecticides to protect stored grains and seeds from insect pests' infestation. Therefore, the present study on evaluation of various extract of *P. pinnata* seed against pulse beetle, *C. chinensis* of green gram was under taken.

MATERIALS AND METHOD

Preparation of extraction

Aqueous Extract:

A weighed quantity of powder passing through a 40-mesh sieve was soaked in water (five parts of water to one part of powder) overnight, shaken thoroughly and filtered through a muslin cloth. The filtrate was diluted with water to prepare 1, 2, 5% concentrations [19,20].

Alcoholic extracts

Cold alcoholic extracts were prepared according to the method Zamon et al 2014 [21]. One gram of powder passed through a 40-mesh sieve was shaken with9 ml of 90% methyl alcohol and allowed to stand for 24 h. It was then filtered through filter paper and washed with the solvent until the filtrate measured 10ml, a 10% extract being thus obtained. This was diluted with water to prepare 1, 2 and 5% concentrations [19, 20].

Petroleum extracts

The powdered seeds were extracted with petroleum ether. 40 gm dried powder of seeds was suspended in 100 ml petroleum ether and keep for 24 h (B.P. 60-80°C). Extract was diluted with tween 80 to prepare 1, 2 and 5% concentrations [18, 19].

Insecticidal efficacy of *P. pinnata* seed extract against insect pests of stored grains.

The pure culture of *C. chinensis* was raised on green gram seeds and maintained under controlled conditions at 27 ±1^{0}C and 70% R.H. The freshly harvested seeds of green gram seeds were sterilized in oven at 50^{o}C for 4 hours[22]. The sterilized grains were put in half kg capacity glass jars and 5 pairs of freshly emerged *C. chinensis* adults were released in the jars. The jars were tightly covered with muslin cloth and were kept in BOD incubator for raising the culture. Petroleum ether extract, methanolic extract and aqueous extracts were used to coat the seeds at the rate of 1, 2 and 5 per cent concentrations. Five pairs of the newly emerged adults of *C. chinensis* were released in each plastic container which was covered with muslin cloth and was tightened with rubber band. The treatments, including control, were maintained in three replications. All the adults were allowed to remain in the container till their natural mortality under room temperature. The mortality of adult beetles released on treated seeds was recorded at 1, 3, 7, 10 and 15 days after treatment till complete kill, even in the control. The egg laying was recorded after 7 and 15 days of release of beetles in each treatment. The adult emergence was monitored at monthly interval for 2 months. The data were analysed in completely randomized block design.

RESULTS

Data in the Table 1. reveal that the different extract of *P. pinnata* seed have caused variable mortality of the adults of *C. chinensis* with maximum mortality in petroleum ether extract and minimum in untreated control. On day-1, maximum mortality (20.22%) was observed in seed coated with petroleum ether extract was statistically at par with methanol extract (15.64%) and Seed coated with aqueous extract resulted 7.50% mortality of the beetles, respectively. The maximum mortality (28.5%) recorded with petroleum ether extract at 5 per cent concentration was statistically at par with methanol extract (22.2%)

On day-3, gave best results (50.56% overall kill) and its 2 and 1 per cent concentrations were equally effective (42.00 and 40.50% mortality). Next equally effective extract were methanolic (45.2%) and aqueous (36.53%) and these were significantly different from rest of the extracts.

day-7, highest mortality (92.63%) *of C. chinensis* was observed in petroleum ether extract coated seeds followed by methanol(76.83%), and aqueous extract (66.86%) all being statistically different from one another. whereas, in untreated control it was 12.22 per cent. Mortality decreased with decrease in concentration of extract. There was complete mortality in petroleum ether coated seeds extract at 5 per cent concentration and was significantly higher than 2 per cent (92.7%) and 1 per cent (85.20%) concentration. In the treatment with methanol and aqueous extract mortality range is not significantly at three tested concentrations.

After 10 days of exposure maximum mortality (100%) was observed in both petroleum ether and methanol extract at 5% concentrations, while at 2 per cent concentration mortality recorded with petroleum ether and methanol extract was 95.0 and 90.7 per cent and both were statistically at par with each other. Mortality recorded with aqueous extract (62.30%) at 5 per cent concentration was statistically at par with their respective lower concentrations (3 and 1%). Maximum mortality

(94%) recorded with petroleum ether extract was statistically at par with methanol extract (87.00%) and both the extract were superior over rest of the treatments

In the 15-day observation, mortality in the control increased substantially to 62.11 per cent and all treatments were superior to the control and no treatment had come at par with petroleum and methanol extract (100.00 and 95.83% kill, respectively). Among tested concentrations, petroleum ether extract at 5 per cent concentration (100.00% kill) was at par with its lower (2 and 1 %) concentrations (both 100.00% kill) and also with petroleum ether at 5 and 2 per cent (100.00%) and aqueous extract at 5 per cent (96.2%) concentration. Methanol and aqueous extract at 2 per cent concentration (100.00 and 80.57%, respectively) was statistically significant with their respective 1 per cent concentration. Among plant extract maximum mortality of the green gram beetle was observed in seeds coated with petroleum ether (71.49%), followed by methanol (64.11%), aqueous (50.71%) all extract differing significantly from one another.

It is evident from Table 2 that on day-7, the minimum number of eggs (4.70 eggs/5 females) was laid by 5 pairs of *C.chinensis* in petroleum ether extract coated seeds followed by methanol (6.60 eggs/5 females) but aqueous extract was less effective (24 eggs/5 females) in preventing oviposition and were statistically at par with each other but was superior to untreated control (104 eggs/5 females).

On day-20, the minimum number of eggs (5 eggs/ 5 females) laid by 5 pairs of beetles on seeds coated with petroleum ether was statistically different from methanol extract (6.60 eggs/ 5 females). Among other treatments, number of eggs observed on seeds treated with aqueous extract (26.94 eggs/ 5females) (Table 3).

Minimum oviposition recorded in green gram seeds treated with petroleum ether (3.60 eggs/ 5 females) and methanol extract at 5 per cent (5.1 eggs/5 females) concentration was statistically at par with its lower concentration except at 1 per cent concentration (12.33 eggs/5 females). The overall picture that emerges from this experiment indicated that petroleum and methanol extract (4.35 and 6.95 eggs/ 5 females) were the best extracts in preventing oviposition. Aqueous extract is less effective (26.19eggs/ 5 females), as compared to other extract.

Data presented in Table 3 reveal that minimum adult emergence (0.55 beetles) was recorded in petroleum ether extract as compared to methanol and aqueous extract 0.85 beetles,1.39 beetle respectively. Progeny production was significantly decreased with the increase in concentration of the petroleum ether(1, 2 and 5%) Petroleum ether and methanol extract at 5 per cent were able to restrict progeny production to 1.30 beetles, and all of them were equally effective. The progeny development in petroleum ether and methanol extract ranged from 0.00 to 2.00 adults/ 5females at all three tested concentrations (5, 2 and 1%).

DISCUSSION

Among the various extracts seeds coated with petroleum ether at 5 per cent concentration caused maximum mortality of the pulse beetle followed by methanol and aqueous extract as compared to control. The results also indicated that comparatively aqueous extract was less effective than petroleum and methanol extract. In this experiment petroleum and methanol extracts were the best extract in preventing oviposition. Similar findings were reported that petroleum ether extract of neem showed ovicidal activity against C. *chinensis* [23, 24, 25] In the present investigation on green gram seed only petroleum ether and methanol extract significantly reduced the expected adult emergence. The adult emergence in petroleum and methanol extract ranged from 0.00 to 2.00 adults/ 5 females at all three tested concentrations (5, 2and 1%). Raghuraman and Singh 1996 [26] reports that petroleum ether extract of neem (at 0.5, 0.75 and 1%) and karanj oil (0.75 and 1%) prevented adult emergence of *C. chinensis* up to 100 days in pigeon pea treated seeds while reduced emergence of pulse beetle adult on cowpea seeds treated with neem and karanj oils at 5 ml/ kg. *P. pinnata* showed insecticidal activity due to presence of secondary metabolites pongamol, pongapin, karanjin, rotenoid, flavone glycosides, flavonoid, etc in it [27,28 ,29]. Karanjin is known as the main active principle in Karanj, which is effective against large number of insects [30]. Out of various extract of *P. pinnata* seed were tested, petroleum ether followed by methanol extract was found to be most effective against pulse beetle, *C. chinensis in* causing mortality, reduced oviposition and adult emergence.

CONCLUSION

Pongamia pinnata is enriched of secondary metabolites; pongamol, pongapin,karangin, rotenoids, flavanoids and fatty acids. Due to these compounds it shows insecticidal activity. Further isolation of active molecule and their insecticidal activity is studied for IPM programme.

Review of Literature

1. Alam M Z.. Pests of stored grain and other stored products and their control. Agricultural Information Service. 1971; 3, R. K. Mission Road, Dhaka, Bangladesh
2. Righi-Assia A F, Khelil M A, Medjdoub BF and Righi K.. Efficacy of oils and powders of someplants in biological control of the pea weevil (*Callosobruchus chinensis* L.). Afr. J. Agric. Res.. 2010; 5: 1474-1481.
3. Booker RH, Observations on three bruchids associated with cowpea in Northern Nigeria. J. Stored Prod Res. 1967; 3: 1-15.
4. Okunola CO. Use of melon oil for the control of bruchid damage in cowpea. In: Proceedings of African Crop Science Society, Ondo State, Nigeria.2003; 6: 238-240
5. Ahmed K.S, Itino T and Ichikawa T. Duration of developmental stages of *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) on azuki bean and the effects of neem and sesame oils at different stages of their development. Pak. J. Bio. Sci. 2003; 6: 932-935.
6. Fishwick FB. Pesticide residues in grain arising from post-harvest treatments. Aspects of Appl. Bio. 1988; 17: 37-46
7. White N DG. Insects, mites and insecticides in stored grain ecosystem, In: Stored grain Ecosystem Ed. D S Jayas, N D G.White and W E. Muir, Marcel Dekker, New York. 1995; pp. 123-168.
8. Tapondjou L A, Adler C , Banda H and Fontem D A. Efficacy of powder and essential oil from Chenopodium ambrosioides leaves as post-harvest grain protectant against six stored products beetle. J. Stored Prod. Res. 2002; 38: 395-402.
9. Gao M, Wang C L S, Zhang S. Irradiation as a phytosanitory treatment for Trogoderma granarium Everts. and Callosobruchus chinensis L. in food and agricultural products. In :Irradiation as a phytosanitory treatment of food and agricultural commodities. Ed. International Atomic Energy Agency, Vienna, Austria . 2004; pp. 75-85.
10. Mishra D, Shukla AK, Tripathi K, Singh K, Dixit A K and Singh K. Efficacy of application of vegetable oils as grain protectant against infestation by *Callosobruchus chinensis* and its effect on milling fractions and apparent degree of dehusking of legumepulses. J. Oleo Sci. 2007; 56: 1-7.
11. Anonymous. The Montreal Protocol on substrates that deplete the ozone layer (with amendments). United Nations Environment Programme, Nairobi, Kenya. Booker.2000

12. Tyler P S, Taylor RW and Rees D P. Insect resistance to phosphine fumigation in food warehouses in Bangladesh. Integrated Pest Control . 1983; 25: 10-13.

13. Collins PJ. Resistance to grain protectants and fumigants in insect pests of stored products in Australia. In: Banks HJ, Wright EJ, Damcevski KA (eds). Stored Grain in Australia. Proceedings of the Australian Post-Harvest Technical Conference. CSIRO: Canberra, Australia. 1998; pp 55–57.

14. Weaveret K, Dunkel F V, Cusker J L and Puyvelde L V. Oviposition patterns in two species of bruchid (Coleoptera: Bruchidae) as influenced by dried leaves Tetrademia riparia, a perennial mint (Lamiales: Lamiaceae) that suppresses population size. Env. Entomol., 1992; 21: 1121-1126.

15. Jilani G M, Khan I and Ghiasuddin N. Studies on insecticidal activity of some indigenous plant materials against the pulse weevil, *Callosobruchus analis* (F.) (Coleoptera: Bruchidae). Pak.J. Entomol. 1988 ; 3: 21-32.

16. Gupta S, Apte S D, and Wast N. Efficacy of some plant material on green gram [Vigna radiata (L.) Wilczek] seed against Callosobruchus maculatus (Fab.). Eur. J. Appl. Sci. 2015; 7: 21–24.

17. Kosar H, and Srivastava M.. Euphorbaceae plant extracts as oviposition deterrent against Callosobruchus chinensis Linn. (Coleoptera: Bruchidae). J. Biopesticide. 2016; 9: 80–90

18. Raghumamu, C P. Insecticidal activity of botanical pesticides on Callosobruchus chinensis L (Coleoptera: Bruchidae) on stored green gram, Vigna radiata (L.). Int. J. Agric. Sci. Res2015; 4: 13–20.

19. Puttarudraih M and Bhatta KL. A preliminary note on studies of Mysore plants as source of insecticides. Indian J. Entomol. 1955; 17:165-169.

20. Deshmukh, SD and Borle, MN. Studies on the insecticidal properties of indigenous plant products. Indian J. Entomol.. 1975; 37: 11-18.

21. Zamin I , Shah JA, Khan I , Majid A , Rehman MM Malik, et al. In-Vitro efficacy of crude extract of Zizipus Jujuba against selected bacterial strains . Int. J. Sci. Res. Pub. 2014 ; 4(2): 1-5.

22. Mookherjee P B, Jotwani, MG., Yadav, T D and Sircar, P. Disinfection of stored seeds by heat treatment. Indian Journal of Entomology. 1968; 27: 476-80.

23. Singh SC. Effect of neem leaf powder on infestation of the pulse beetle, *C. chinenesis* in stored Khesari. Indian J Ent. 2003; 65(2)188-192.

24. Vir S. 1994. Effectiveness of some natural oils in protection of cowpea seeds from bean weevil Callosobruchus maculatus. Bulletin of Grain Technol. 32: 68-91

25. Khaire VM., Kachare B V and Mote U N. Efficacy of different vegetable oils as grain protectants against pulse beetle, Callosobruchus chinensis L. in increasing storability of pigeonpea. J. Stored Prod. Res. 1992;28: 153-156

26. Raghuraman, S and Singh D. Biopotentials of Azadirachta indica and Cedrus deodara oils on Callosobruchus chinensis. Int. J. Pharmacognosy. 1997; 35: 344-348

27. Shameel, S., Usmanghani, K., Ali, M.S., Ahmad, V.U. Chemical constituents from the seeds of *Pongamia pinnata* (L.) Pierre. Pak J Pharm Sci. 1996; 9(1):11-20.

28. Simin, K, Zulfiqar A, Sayed Muhammad Khalid – UZ – Zaman, and Ahmad VU. Structure and biological activity of a new rotenoid from *Pongamia pinnata*. Nat. Prod. Letters, 2002;16 (5): 351-357.

29. Chauhan D. and Chauhan JS. Flavonoid glycosides from *Pongamia pinnata.* Pharmaceutical Bio. 2000; 40 (03): 171–174.

30. Mathur,YK, Srivastava JP, Nigam SK and Banerji R. Juvenomimetic effects of karanjin on the larval development of flesh fly Sarcophaga ruficornis (Cyclorrhapha: Diptera). J. Ent. Res. 1990; 14(1): 44-51.

Table 1. Effect of *P. pinnata* seed extracts against mortalility of green gram of *C.chinensis*

Treatments	mortality (%) of beetle at indicated days and concentrations											
	day 1				day 3				day 7			
	Concentration				Concentration				Concentration			
	5.00%	2.00%	1.00%	Mean	5.00%	2.00%	1.00%	Mean	5.00%	2.00%	1.00%	Mean
PE	28.5 ±0.21	22.16 ±0.11	10.4 ±0.12	20.22 ±0.23	70.56 ±0.23	42.00 ±0.35	39.33 ±0.14	50.63 ±0.15	100 ±0.10	92.7 ±0.11	85.2 ±0.08	92.63 ±0.16
ME	20.22 ±0.11	16.70 ±0.26	10.0 ±0.16	15.64 ±0.20	65.00 ±0.11	40.50 ±0.60	30.30 ±0.11	45.2 ±0.10	85.4 ±0.41	76.2 ±0.09	68.9 ±0.36	76.83 ±0.11
AE	12.50 ±0.05	6.50 ±0.24	3.50 ±0.33	7.5 ±0.41	54.9 ±0.16	34.20 ±0.24	20.50 ±0.13	36.53 ±0.21	75.2 ±0.13	65.4 ±0.1	60.0 ±0.23	66.86 ±0.21
Control	0.00 ±0.00	0.00 ±0.00	0.00 ±0,00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	5.00 ±0.10	10.00 ±0.12	12.22 ±0.11	9.07 ±0.11

Treatments	mortality (%) of beetle at indicated days and concentrations								
	Day-10				Day-15				Over all mean mortality
	Concentrations				Concentrations				
	5%	2%	1%	Mean	5%	2%	1%	Mean	
PE	100 ±0.01	95 ± 0.03	87 ±0.04	94 ±0.02	100 ±0.04	100 ±0.06	100 ±0.03	100 ±0.04	71.49 ±0.05
ME	95.56 ±*0.02*	90.7 ±0.03	75 ±0.05	87.08 ±0.33	100 ±0.14	100 ±0.11	87.2 ±0.11	95.83 ±0.12	64.11 ±0.12
AQ	83.2±0.11	72.4±0.08	62.3±0.65	61.86±0.26	96.2±0.23	80.57±0.15	65.7±0.17	80.83 ±0.26	50.716±0.26
control	35.3 ±0.22	30.00 ±0.16	30.00 ±0.27	31.7 ±0.23	66.2 ±0.34	60.2 ±0.12	60.0 ±0.23	62.1 ±0.23	20.574 ±0.23

PE= Petroleum ether Extract ME= Methanol Extract AQ= Aqueous Extracts

Table 2 Effect of *P. pinnata* seed extract against oviposition by *C. chinensis* on treated green gram seeds

treatments	Number of egg laid/5 pairs of beetles at different days and concentrations								
	Days -7				Days-20				Over all mean number of egg laid/5 pairs of beetles
	concentration				concentration				
	5%	2%	1%	Mean	5%	2%	1%	Mean	
PE	3.60 ±0.13	4.9 ±0.11	5.60 ±0.32	4.70 ±0.16	3.0 ±0.35	5.35 ±0.22	6.00 ±0.12	4.00 ±0.23	4.35 ±0.13
ME	4.67 ±0.11	6.67 ±0.42	8.67 ± 0.21	6.60 ±0.19	5.1 ±0.12	7.60 ±0.21	9.30 ±0.31	7.30 ±0.31	6.95 ±0.16

Aq	12.33 ±0.15	23.00 ±0.14	35.50 ±033	26.94 ±0.27	13.00 ±0.13	25.00 ±0.14	38.2 ±0.17	25.44 ±0.13	26.19 ±0.13
Control	110.33 ±0.07	97.3 ±0.11	105 ±0.22	104.21 ±0.13	113.2 ±0.11	99.2 ±0.13	104.9 ±0.05	105.7 ±0.09	104.95 ±0.07

PE= Petroleum ether Extract ME= Methanol Extract AQ= Aqueous Extract

Table 3 Effect of *P. pinnata* seed Extract on number of beetles/5 pairs developed after 60 days of treatment of green gram seeds at different concentration

treatments	**Mean number of beetle /5 pairs developed at indicated concentration**			
	5%	**2%**	**1%**	**Mean**
Petroleum ether	0.00 ±0.11	0.32±015	1.30±0.13	0.55±0.13
methanol	0.00±0.01	0.59±0.11	1.98±0.05	0.85±0.05
Aqueous	0.30±0.03	1.32±0.12	2.56±0.05	1.39±0.66
Control	45.22±0.02	50.10 ±0.13	52.22±0.16	49.18±0.10