

## **Comparative performance on insecticidal and oviposition deterrence of cashew nut shell liquid (CNSL) on bruchids (*Callosobruchus chinensis* L.) in cowpea (*Vigna unguiculata* (L.) Walp.) seed**

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### **ABSTRACT**

Cowpea (*Vigna unguiculata* (L.) Walp) seed is greatly affected by the pest called bruchids (*Callosobruchus* sp.) during storage. Search for organic based biopesticide for control of bruchids led to the use of Cashew Nut Shell Liquid (CNSL) that had an invigorating effect on maintenance of seed viability. The seeds treated with CNSL recorded higher germination in 12 months storage. Number of eggs, number of insects and seed damage percentage were meagre in CNSL treated seeds but was comparatively effective to neem oil.

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### **INTRODUCTION**

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important pulse crop in which the seed is mainly affected by the storage pest called pulse beetle or bruchid (*Callosobruchus* sp.). It is estimated that this pest causes huge loss - between 20 and 50% - on stored cowpea and sometimes the loss could be complete accounting for 100% because of its short life cycle and high reproductive capacity (Shaaya *et al.*, 1997; Udo and Harry, 2013; Mogbo *et al.*, 2014; Tufail *et al.*, 2015). The beetle starts demolition drive right from the field prior to harvest through storage where the insect population builds up to damaging levels (Patnaik *et al.*, 1986; De Lima, 1987; Baidoo *et al.*, 2010). It lays egg on the surface of the seed and the egg hatches when congenial conditions become available.

The beetle can be controlled by seed treatment with pesticides that can alleviate the problem and are cost effective (Paul *et al.*, 1996; Abd El-Razik and Zayed, 2014). However, indiscriminate and repeated use has created problems like

pesticide residues, pest resurgence, development of pesticide resistance, secondary pest outbreak and also deleterious effect on non target or beneficial organism (Katyal and Satake, 1996; Wahedi *et al.*, 2015). Alternatively plant products such as coconut oils, plant extracts and botanicals have proved their effectiveness in controlling the pulse beetle (Elhag, 2000; Tandon *et al.*, 2004; Omotoso, 2008; Rahman *et al.*, 2010; Ilboudo *et al.*, 2010; Yusuf *et al.*, 2011; Rohan and Disna, 2012; Wahedi *et al.*, 2015; Albandari, 2015; Tufail *et al.*, 2015). Cashew Nut Shell Liquid (CNSL) is one such plant product, with oviposition deterrence effect on bruchids (Raja, 2008; Raja *et al.*, 2013; Raja *et al.*, 2015). It is a by-product of cashew industry which is extracted by pressing the shells or using organic solvents. It contains approximately 70% anacardic acid, 18% cardol and 5% cardanol, with the remainder being made up of other phenols and less polar substances (Tyman and Kiong, 1978; Lubic and Thachil, 2003). Therefore, the research was

carried out to control the pulse beetle by using the CNSL as an alternative plant product.

The United States Environmental Protection Agency Report on CNSL indicated that the distilled CNSL has been shown to be biodegradable when tested using OECD Method 302D (96% degradation after 28 days) in a GLP study. This indicates that the liquid can be used for seed treatment and it will be degraded when the seed is sown in the soil. Hence, a study was conducted to assess the oviposition deterrence effect of CNSL on bruchid and its comparative performance in cowpea (*Vigna unguiculata* (L.) Walp.) seed.

### **MATERIALS AND METHODS**

Generally the shell of the cashew is formed of three concentric layers viz., epicarp, mesocarp and endocarp. The mesocarp of the nut is spongy and is made up of a mass of sclerenchymatic tissues and ducts containing about 35% of a sticky, resinous liquid called CNSL. This CNSL was extracted by compressing the shell through a mechanical press and filtered for further use. Coconut oil was obtained by the mechanical extraction and filtration similar to CNSL. The TNAU neem oil 60 EC was obtained from the Tamil Nadu Agricultural University, Coimbatore. The malathion 5% dust formulation manufactured by the Gujarat Pesticides Limited and TATAMONO, the monocrotophos chemical by the Rallis India Limited were used for the seed treatment.

#### **Seed treatment with CNSL and seed storability**

The experiment was conducted at the Department of Seed Science and Technology, Agricultural College and Research Institute, Madurai. Freshly harvested cowpea seeds were collected and treated with cashew nut shell liquid at various doses as per the treatment details viz., T<sub>1</sub> - Untreated control, T<sub>2</sub> - CNSL @ 1 mL kg<sup>-1</sup>, T<sub>3</sub> - CNSL @ 2 mL kg<sup>-1</sup>, T<sub>4</sub> - CNSL @ 3 mL kg<sup>-1</sup>, T<sub>5</sub> - CNSL @ 4 mL kg<sup>-1</sup>, T<sub>6</sub> - CNSL @ 6 mL kg<sup>-1</sup>, T<sub>7</sub> - CNSL @ 8 mL kg<sup>-1</sup>, T<sub>8</sub> - CNSL @ 10

mL kg<sup>-1</sup>, T<sub>9</sub> - CNSL @ 15 mL kg<sup>-1</sup> and T<sub>10</sub> - CNSL @ 20 mL kg<sup>-1</sup>. The treated seeds were stored in polythene bags at ambient room temperature. The seed samples of all the treatments were drawn at two months interval for the evaluation of the seed viability and vigour. The germination test was conducted with 400 seeds in four replications (ISTA, 1999). The vigour index was calculated by following the formula, vigour index = germination percentage x seedling length (cm) (Abdul-Baki and Anderson, 1973).

#### **Comparative study of CNSL with plant oils and chemicals**

Based on the results obtained from the earlier experiment, the optimum dose for cowpea seed treatment was fixed at 3 mL kg<sup>-1</sup> of seed. Freshly harvested cowpea seeds were treated with the CNSL and other products as per the details viz., T<sub>1</sub> - Untreated control, T<sub>2</sub> - CNSL @ 3 mL kg<sup>-1</sup>, T<sub>3</sub> - Neem oil 60 EC @ 5 mL kg<sup>-1</sup>, T<sub>4</sub> - Coconut oil @ 5 mL kg<sup>-1</sup>, T<sub>5</sub> - Activated clay @ 10 g kg<sup>-1</sup>, T<sub>6</sub> - Malathion 5% Dust @ 5 g kg<sup>-1</sup>, T<sub>7</sub> - Monocrotophos 36 SL @ 4 mL kg<sup>-1</sup> of seed and stored in polythene bags and kept under ambient storage conditions. Germination test was conducted with 400 seeds in four replications (ISTA, 1999) at bimonthly intervals.

Five pairs of adult beetles per kilogram were introduced in another set of above detailed treated seeds for the assessment of the toxicity and oviposition deterrence of CNSL and other products. About 100 seed samples were drawn on 75<sup>th</sup> day in each treatment and assessed for ovipositioning on the seed surface. The seed infestation percentage was scrutinized by observing the presence of exit holes. The data generated were subjected to analysis of variance (ANOVA) at (0.05) significant level and Duncan's Multiple Range Test was used for means separation (Panse and Sukhatme, 1967).

**Table 1.** Effect of cashew nut shell liquid treatment on viability of cowpea seed during storage

Treatments	Germination (%)							
	Initial	2 MAS	4 MAS	6 MAS	8 MAS	10 MAS	12 MAS	Mean
T1- Untreated seed	94	86	88	85	66	64	60	77.6
T2- CNSL @ 1 mL kg <sup>-1</sup>	95	85	85	85	70	70	69	79.8
T3- CNSL @ 2 mL kg <sup>-1</sup>	95	90	84	83	73	70	68	80.4
T4- CNSL @ 3 mL kg <sup>-1</sup>	95	91	90	89	80	80	80	86.4
T5- CNSL @ 4 mL kg <sup>-1</sup>	92	80	82	82	79	79	73	81.0
T6- CNSL @ 6 mL kg <sup>-1</sup>	91	80	81	81	75	75	73	79.4
T7- CNSL @ 8 mL kg <sup>-1</sup>	91	84	80	79	78	78	65	79.3
T8- CNSL @ 10 mL kg <sup>-1</sup>	91	84	81	81	80	76	71	80.6
T9- CNSL @ 15 mL kg <sup>-1</sup>	91	80	74	77	76	76	75	78.4
T10- CNSL@20 mL kg <sup>-1</sup>	92	84	81	81	79	77	75	81.3
Mean	92.7	84.4	82.6	82.3	75.6	74.5	70.9	
	S	T	SxT					
SEd	1.0	1.2	3.3					
CD (P=0.05)	2.1	2.5	6.6					

(\*MAS-Months after storage; S- Storage period; T - Treatment)

**Table 2.** Effect of cashew nut shell liquid treatment on seedling vigour in cowpea seed during storage

Treatments	Vigour index							
	Initial	2 MAS	4 MAS	6 MAS	8 MAS	10 MAS	12 MAS	Mean
T1- Untreated seed	3146	2808	3124	3005	2075	1920	2172	2607
T2- CNSL @ 1 mL kg <sup>-1</sup>	3674	2657	2801	3369	2012	2264	2505	2754
T3- CNSL @ 2 mL kg <sup>-1</sup>	3213	2844	2785	3357	2033	2194	2503	2704
T4- CNSL @ 3 mL kg <sup>-1</sup>	3503	2875	3027	3489	2264	2776	2874	2972
T5- CNSL @ 4 mL kg <sup>-1</sup>	3051	2512	2928	2923	2172	2456	2701	2677
T6- CNSL @ 6 mL kg <sup>-1</sup>	2993	2304	2790	3086	2162	2476	2717	2647
T7- CNSL @ 8 mL kg <sup>-1</sup>	3116	2566	2304	3084	2332	2624	2405	2702
T8- CNSL @ 10 mL kg <sup>-1</sup>	3025	2895	2566	3329	2340	2519	2633	2787
T9- CNSL @ 15 mL kg <sup>-1</sup>	3116	2536	2895	3087	2332	2599	2643	2695
T10- CNSL@20 mL kg <sup>-1</sup>	3330	2906	2536	3098	2414	2425	2748	2839
Mean	3217	2690	2852	3182	2213	2425	2590	
	S	T	SxT					
SEd	62.3	74.4	197.0					
CD(P=0.05)	124.2	148.5	393.0					

(\*MAS-Months after storage; S- Storage period; T - Treatment)

## RESULTS AND DISCUSSION

### Seed treatment with CNSL and seed storability

Experimental results showed that the cowpea seeds treated with CNSL have a significant effect on its viability and vigour during 12 months storage. The seeds treated with the lower doses *viz.*, 1, 2 and 3 mL kg<sup>-1</sup> have showed stimulatory effect on germination and seedling vigour during initial period. However,

the seeds treated with CNSL @ 3 mL kg<sup>-1</sup> significantly (P=0.05) maintained the higher germination (df=7, F=7.2) and vigour (df=9, F=4.0) during 12 months storage when compared to other doses and untreated control (Table 1 & 2). The increase in the concentrations leads to slight decrease in germination and seedling vigour during initial evaluation. Also, the lower or higher doses have resulted in lesser germination percentage during

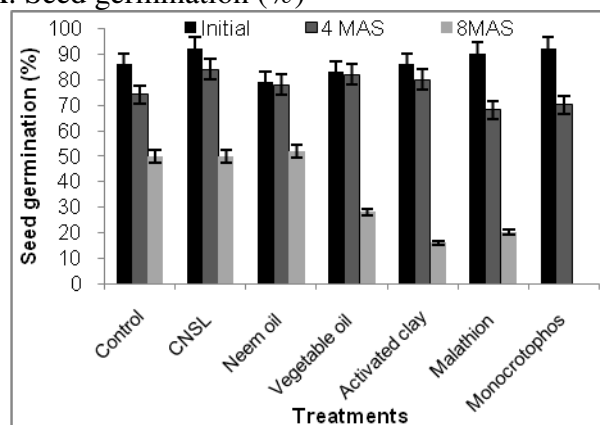
12 months storage. Mean germination percentage showed similar trend of higher germination in CNSL @ 3 mL kg<sup>-1</sup> treated seeds during the entire storage period. However, decline in germination from 92.7 per cent to 70.9 per cent (df=7, F=7.2, P=0.05) was observed irrespective of concentrations which were mainly due to the ageing process of the seeds.

Computed vigour index showed similar results in which the maximum index (2874) was recorded in the CNSL @ 3 mL kg<sup>-1</sup> treated seeds at 12<sup>th</sup> month of storage. The mean values recorded over the storage period indicated similar trend of vigour maintenance in the above prescribed concentration than the others and control (Table 2). Therefore, it was concluded that the CNSL @ 3 mL kg<sup>-1</sup> of seed would be effective for seed viability (df=7, F=7.2, P=0.05) and vigour (df=9, F=4.0, P=0.05) maintenance in cowpea. Raja (2008) found that the CNSL dose of 4 mL kg<sup>-1</sup> had a better effect on viability maintenance in blackgram seeds. The slight differences in dose and its effect on seed quality were decided by the surface area of the seeds. Similar results of viability and vigour maintenance by seed treatment with plant oils and insect control were proved in legumes and pulses by several workers (Lele and Mustapha, 2000; Songa and Rono, 2010; Yusuf *et al.*, 2011; Raja *et al.*, 2013; Asawalam and Anaeto, 2014; Raja *et al.*, 2015; Wahedi *et al.*, 2015). The seed treatment with resinous CNSL acted as a coating material on the surface of the seed which might prevent moisture entry into seed. This might lead to reduction in the ageing process of the seed. The fungicidal property of the CNSL (Mandal, 1997) might also be the reason for the maintenance of viability and vigour.

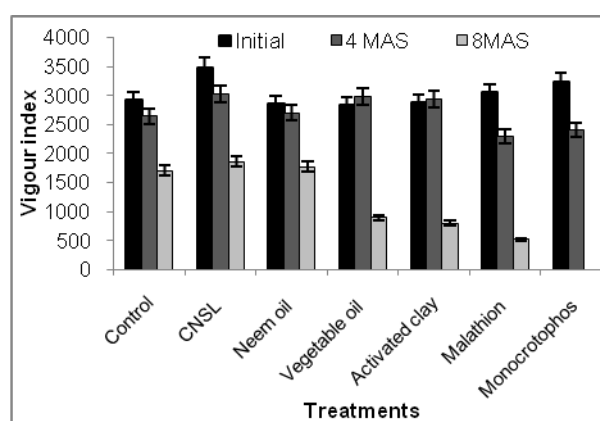
#### Comparative study of CNSL with plant oils and chemicals

Comparative efficacy of the different seed treatments revealed initial significant differences in cowpea seed germination. Initially the higher germination of 92 per cent was observed in CNSL and monocrotophos treated seeds. However, the seeds lost their viability during storage, irrespective of the treatments.

#### A. Seed germination (%)



#### B. Vigour index

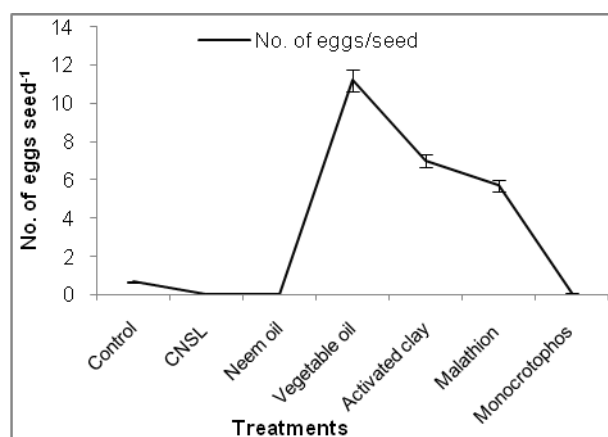


**Fig 1.** Comparative efficacy of cashew nut shell liquid and other seed treating chemicals on storability of cowpea seed.

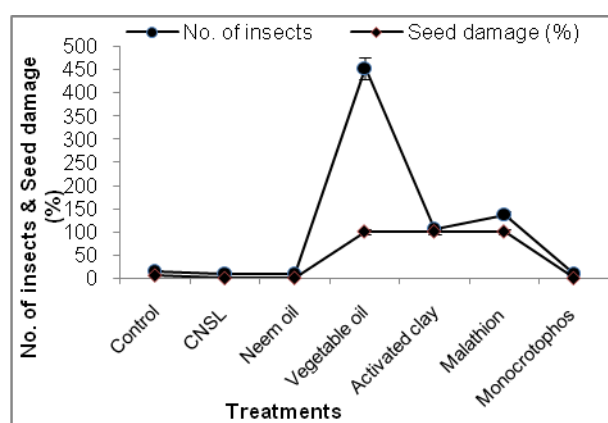
Poor performance in viability retention was noticed in vegetable oil, activated clay, malathion and monocrotophos treatments. Germination of 52 and 50 per cent was observed in neem oil and CNSL treated seeds respectively and therefore, these treatments comparatively performed better than others (Fig. 1A) during eight months storage.

Seedling vigour index showed similar trend like viability maintenance in which the CNSL treated seeds have recorded higher index among the treatments (Fig. 1B). Higher number of eggs was observed on the surface of the vegetable oil treated seeds which ultimately resulted in higher beetle population and infestation percentage are shown in Fig. 2 A and B.

## A. Number of eggs on the seed



## B. Number of insects and seed damage (%)



**Fig 2.** Comparative efficacy of cashew nut shell liquid and other seed treating chemical on bruchid infestation in cowpea seed.

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antifungal, antibacterial and antiviral properties (Harikrishnan *et al.*, 2003; Sharma and Singh, 2014) and this compound is active in nearly 550 insect species including pulse beetle (Anuradha and Annadurai, 2008) which might be the reason for the lesser insect multiplication and seed damage percentage.

Raja (2008) found that the CNSL had both toxic and oviposition deterrence effect in blackgram seed as it caused low adult emergence, egg laying and percentage seed infestation. He opined that the reduced egg laying might be due to the oily nature, toxic substances or repellent compounds which caused altered insect behaviour. Generally CNSL is bitter, caustic and fumigatory with smokes that irritate and gives off choking fumes (Raja *et al.*, 2013). This might be the reason for oviposition deterrence and low adult emergence in cowpea seed. Mandal (1997) reported that the cardol content of CNSL has pronounced insecticidal properties as well as providing excellent preservative effect on timbers, books and stationery especially to prevent the attack of insects. Nevertheless, this insecticidal property might also caused lower adult built up and seed infestation percentage in cowpea seed.

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However, no egg was noticed in CNSL and neem oil treated seeds which led to no seed damage. Monocrotophos treated seeds also showed similar kind of better performance in insect infestation. The study clearly indicates that neem oil and CNSL are as effective as insecticides in bruchid control and these oils are from plant origin which will not cause any residual effect on the seed. Similar trends of damage (Mogbo *et al.*, 2014) and insect population control by seed treatment with neem product (Tufail *et al.*, 2015) was recorded in cowpea.

Several workers have found the effectiveness of neem oil on the control of pulse beetle during seed storage (Ilesanmi and Gungula, 2010; Raja *et al.*, 2013; Wahedi *et al.*, 2015). It is reported that the presence of active compound called azadirachtin in neem oil has insecticidal,

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