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Safety evaluation of cashew nut shell liquid formulations

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Abstract
The Cashew nut shell liquid based emulsifiable concentrate botanical formulation at effective (0.3%) and above effective doses (0.6%) proved to be safe and the application does not affected the crop adversely, indicated its safety with no phytotoxicity symptoms on cowpea as per phytotoxicity rating scale of CIBRC. The bioassay study on the effect the CNSL formulation on the predator *Coccinella transversalis* Fabricius and the parasitoid *Bracon* sp under laboratory conditions by dry film technique showed no significant adverse effect on the natural enemies.

Keywords: CNSL formulation, safety, phytotoxicity, coccinellids

Introduction
The use of plant secondary metabolites in pest management is gaining importance in present day agriculture in order to reduce the pesticide load on the ecosystem. An alternative organic pesticide to the one and only commercial botanical neem formulation is highly essential in the current scenario. Cashew nut shell liquid is one among the above said category and is not explored for its potential in agriculture, though it has been widely employed in polymer industry. The Phukerd and Soonwera (2013) [10] reported that secondary metabolites are phytochemicals which are biosynthetically derived from primary metabolites and act against insect defense mechanisms. CNSL, the most known source of phenolic lipids in plants and can replace phenols in many situations. The oil mainly composed of four phenolic constituents – hydroxyalk (en) ylbenzene homologs *viz*., anacardic acid, cardanol, cardol and 2-methyl cardol. Unlikely to other phenolic lipids found in cereals, the chain length of these constituents is not varying. However, each of them composed of a mixture of four components differed by their degree of unsaturation (Kumar *et al*., 2002) [6]. The effectiveness of CNSL and cashew nut shell extract against sucking pests was indicated in the study of Oparaeke *et al*. (2005) [9] wherein the extracts of cashew nut shell + garlic bulb and cashew nut shell + African pepper mixed in the ratio of 10:10% w/w were effective in reducing the cowpea pests *viz*., flower bud thrips and pod sucking bugs. The efficacy of 1% ethanolic extract of CNSL in protecting the pods of cowpea, *V. unguiculata* against field insect pests has been reported by Olotauh and Ofuya (2010) [8]. In this context, a study regarding the phytotoxicity of CNSL based botanical formulation and its safety to natural enemies is aimed in the present study.

Materials and Methods
Cashew nut shell liquid based EC formulations were prepared using appropriate solvents and emulsifiers with 20 percent active ingredient of CNSL (EC-1) and 20 percent CNSL in combination with 20 percent pongamia oi (EC-2) l and evaluated in field for its phytotoxicity and laboratory trial for safety to natural enemies. The safety of emulsifiable concentrate formulations of CNSL, EC-1 (20% CNSL) and EC-2 (20% CNSL + 20% pongamia oil) at effective doses (0.1 and 0.3%) and double the effective doses (0.2 and 0.6%) were evaluated on the crop, predators, parasitoids and pollinators. The following treatments were applied with three replications.

The following treatments were applied with three replications

Recommended dose	Double the recommended dose
T1: 0.1% CNSL EC-1	T1: 0.2% CNSL EC-1
T2: 0.3% CNSL EC-1	T2: 0.6% CNSL EC-1
T3: 0.1% CNSL EC-2	T3: 0.2% CNSL EC-2
T4: 0.3% CNSL EC-2	T4: 0.6% CNSL EC-2
T5: Blank formulation	T5: Blank formulation
T6: Pongamia oil 1%	T6: Pongamia oil 1%
T7: Neem oil garlic emulsion 2%	T7: Neem oil garlic emulsion 2%
T8: Thiamethoxam 25 WG 0.03%	T8: Thiamethoxam 25 WG 0.03%
T9: Untreated control	T9: Untreated control

Phytotoxicity on Cowpea, *V. unguiculata* sub sp. *sesquipedalis*: Cowpea, *V. unguiculata sesquipedalis* variety Vellayani Jyothika was raised at Farming systems Research Station, Sadanandapuram. Plots of 4 x 4 m^2 were prepared and four pits were taken at 2m apart and sown with seeds of cowpea (Plate 1). The excess seedlings were thinned two weeks after germination and only two seedlings were maintained in a pit. The plants were trailed over nylon net placed over the GI wire tied between poles erected along rows of plants. The nylon net was cut in such a fashion to form a 0.25 m^2 around each plant on to which plants were individually trailed. The crop was maintained as per the recommended package of practices of Kerala Agricultural University (KAU, 2016) [4].

Plate 1: Experimental field

The treatments were applied to the plants at 30 and 60 DAP using a hand sprayer to the point of run off. The plants were visually scored for phytotoxic symptoms *viz*., injury to leaf tip, injury to leaf surface, wilting, vein clearing, necrosis, epinasty and hyponasty, individually as per the protocol of Central Insecticide Board Registration Committee (CIBRC) at 1, 3, 7, 10, 14 and 21 days after spraying in the 0 to 10 scale mentioned below.

Individually as per the protocol of (CIBRC) at 1, 3, 7, 10, 14 and 21 days after spraying in the 0 to 10 scale mentioned below.

Rating	Phytotoxic symptoms (%)
0	No symptom
1	1-10
2	11-20
3	21-30
4	31-40
5	41-50
6	51-60
7	61-70
8	71-80
9	81-90
10	91-100

$$\text{Percent leaf injury} = \frac{\text{Total grade points}}{\text{Max. grade x No. of leaves observed}} \times 100$$

Safety to Predators and Parasitoids

Toxicity of EC-1 and EC-2 at effective and double the effective doses to the coccinellid predator, *Coccinella transversalis* was evaluated in the laboratory by dry film technique developed by McCutchen and Plapp (1988) [6] and modified by Chelladurai (1999) [2]. The adults of *C. transversalis* were collected from the cowpea fields and maintained in the laboratory for egg laying. *A. craccivora* was provided as food. A tissue paper with multifold was also hung inside the jar for oviposition (Gautam, 2018). Egg masses laid on tissue paper were removed gently using a sharp blade and kept for further development. The eggs which were about to hatch (that appear black with developing embryo) was placed in plastic container along with aphids as prey to prevent cannibalism and reared undisturbed for 2-3 days. The predators were reared in the laboratory at 27±10 C and 60±5% RH. These containers were checked daily and food was provided until the grubs attained pre-pupal stage. The pupae were left undisturbed until emergence. Newly emerged adults were fed on aphids and were used for the experiment.

0.5 mL of each treatment was pipetted out individually to the glass tube of 15 x 2.5 cm size, which was constantly rotated and rolled on a flat surface so that the pesticide forms a thin uniform coating inside wall of test tube. For untreated check, water was used. After drying the tubes, test insects were released at the rate of 10 per vial and were provided with aphids as food. Tubes were covered with muslin cloth and secured with rubber bands Each tube served as one replication. Mortality of the predators was taken at 1, 3 and 5 days after treatment and subjected to statistical analysis.

The *B. brevicornis* were procured from the Parasite Breeding Station, Parottukonam, Thiruvananthapuram. Dry film technique was adopted for laboratory assay. Adult parasitoids (10 numbers per replication) were released to treated tubes and provided with honey solution as food. The mortality was recorded at 1, 3 and 5 days after treatment and subjected to statistical analysis.

Results

Phytotoxicity evaluation

The emulsifiable concentrate CNSL formulations, EC-1 and EC-2 were evaluated for its phytotoxicity on cowpea plants by spraying at effective and double the effective dose and scored for phytotoxic symptoms *viz*., yellowing, scorching, necrosis, epinasty and hyponasty at different intervals *ie*., 0, 1, 3, 5, 7, 10 and 15 days after spraying. The results revealed that the application of applications of different concentrations of EC-1 and EC-2 at effective dose (0.1 and 0.3%) and double

the effective dose (0.2 and 0.6%) did not have any adverse effect on the crop with no phytotoxic symptoms (Table 1). However the treatments with 0.6% of EC-1 and EC-2 produced some necrotic symptom in the form of minute black spots (10 percent) with phytotoxicity rating of 1.

Table 1: Phytotoxicity evaluation of CNSL formulations on cowpea

Treatments	Concentration of CNSL		Phytotoxic symptoms									
			Yellowing		Necrosis		Scorching		Epinasty		Hyponasty	
	E	AE	E	AE	E	AE	E	AE	E	AE	E	AE
EC-1	0.1	0.2	0	0	0	0	0	0	0	0	0	0
	0.3	0.6	0	0	0	1	0	0	0	0	0	0
EC-2	0.1	0.2	0	0	0	0	0	0	0	0	0	0
	0.3	0.6	0	0	0	1	0	0	0	0	0	0
Blank formulation			0		0		0		0		0	
Pongamia oil 1%			0		0		0		0		0	
Neem oil garlic emulsion 2%			0		0		0		0		0	
Thiamethoxam 25 WG 0.03%			0		0		0		0		0	
Untreated control			0		0		0		0		0	

CNSL- Cashew Nut Shell Liquid, EC-1 -20% CNSL, EC-2 – 20% CNSL + 20% pongamia oil
E- Effective dose AE- Above effective dose

Safety of CNSL Formulations to the Predator

In order to recommend the developed formulations to field, the safety of the formulations need to be assessed against the natural enemies at effective and the above effective dose. The results of the screening of CNSL formulations, EC-1 and EC-2 against coccinellid predator, *Coccinella transversalis* are presented in table 2. At the effective dose, (0.1 and 0.3%) no mortality of *C. transversalis* was observed in any of the treatments except chemical check thiamethoxam 0.03% (16.67 percent) at 1 DAT. At above effective dose (0.6%), both EC-1 and EC-2 recorded 3.33 percent mortality each. Mortality ranging from 3.33 to 10.00 percent at the effective dose and 10.00 to 13.33 percent at above effective dose of CNSL formulations, EC-1 and EC-2 was observed at 3 DAT. This mortality was comparable to the mortality caused by other botanical pesticides neem oil garlic emulsion 2% (6.67 percent) and pongamia oil 1% (3.33 percent) and significantly less than that of chemical check thiamethoxam 0.03% (46.67 percent). At 5 DAT, the mortality caused by the CNSL formulations at effective dose ranged from 10.00 to 20.37 percent and was on par with botanicals neem oil garlic 2% (13.7 percent) and pongamia oil 1% (17.41 percent). However at above effective dose of 0.6% CNSL, the toxicity ranged from 31.11 to 37.78 percent mortality which was significantly different that of botanicals. The mortality caused by CNSL formulations were significantly less than that of chemical check, thiamethoxam 0.03%.

Table 2: Mortality of *Coccinella transversalis* treated with different doses of CNSL formulations

Treatments	CNSL Concentration		Percent mortality*					
			1 DAT		3 DAT		5 DAT	
	E	AE	E	AE	E	AE	E	AE
EC-1	0.1	0.2	0.00 (0.00)	0.00 (0.00)	3.33 (6.14)	10.00 (18.43)	10.00 (14.99)	30.74 (33.51)
	0.3	0.6	0.00 (0.00)	3.33 (6.14)	6.67 (12.29)	13.33 (21.14)	13.33 (17.21)	31.11 (33.88)
EC-2	0.1	0.2	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	13.33 (21.14)	10.00 (14.99)	30.74 (33.51)
	0.3	0.6	0.00 (0.00)	3.33 (6.14)	10.00 (18.43)	13.33 (21.14)	20.37 (26.41)	37.78 (37.89)
Blank formulation			0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Pongamia oil 1%			0.00 (0.00)	0.00 (0.00)	3.33 (6.14)	3.33 (6.14)	17.41 (24.37)	17.41 (24.37)
Neem oil garlic emulsion 2%			0.00 (0.00)	0.00 (0.00)	6.67 (12.29)	6.67 (12.29)	13.70 (21.48)	13.70 (21.48)
Thiamethoxam 25 WG 0.03%			16.67 (23.85)	16.67 (23.85)	46.67 (43.06)	46.67 (43.06)	96.67 (83.85)	96.67 (83.85)
SE(m)			0.958	3.217	4.396	3.556	5.966	3.087
CD (0.05)			(2.896)	(9.728)	(13.293)	(10.753)	(18.041)	(9.334)

CNSL- Cashew Nut Shell Liquid, EC-1 -20% CNSL, EC-2 – 20% CNSL + 20% pongamia oil,
E- Effective dose AE - Above effective dose *Corrected with Abbot's formula over control,
Mean of 10 adults per treatment with tree replication Figures in the parenthesis are angular transformed values, DAT- Days after treatment

Toxicity of CNSL Formulations to the Parasitoid

The toxicity of the developed formulations, EC-1 and EC-2 was also tested against the parasitoid, *Bracon* sp (Table 3).

At the effective dose, (0.1 and 0.3%) no mortality of *Bracon* sp was observed in 0.1% EC-1 and EC-2 and 0.3% EC-1, but a 6.67 percent mortality was recorded with 0.3% EC-2 and NOG 2% each. This was followed by pongamia oil 1% with 3.33 percent mortality. All the above treatments produced significantly lower mortality than chemical check thiamethoxam 0.03% (33.33 percent) at 1 DAT. At above

effective dose, EC-1 did not produce any mortality but EC-2 recorded 3.33 percent mortality each was on par with NOG 2%.

Mortality ranging from 3.70 to 14.81 percent at the effective dose and 10.37 to 24.81 percent at above effective dose of CNSL formulations, EC-1 and EC-2 was observed at 3 DAT. This mortality was significantly lower compared to other botanical pesticides neem oil garlic emulsion 2% (25.92 percent) and significantly less than that of chemical check thiamethoxam 0.03% (62.96 percent).

At 5 DAT, the mortality caused by the CNSL formulations at effective dose ranged from 22.62 to 31.54 percent. 0.1 and 0.3% EC-1and 0.1% EC-2 caused a mortality lower than the botanical NOG 2% but was on par with pongamia oil 1%. However at above effective dose of 0.2 and 0.6% CNSL, the toxicity ranged from 28.57 to 33.33 percent mortality which was at par with botanical pesticides NOG 2% (33.33 percent) and pongamia oil 1%(19.04 percent). The mortality caused by CNSL formulations was significantly less than that of chemical check, thiamethoxam 0.03%.

Table 3: Mortality of *Bracon* sp treated with different doses of CNSL formulations

Treatments	CNSL Concentration		Percent mortality*					
			1 DAT		3 DAT		5 DAT	
	E	AE	E	AE	E	AE	E	AE
EC-1	0.1	0.2	0.00 (0.00)	0.00 (0.00)	3.70 (6.49)	14.07 (21.83)	22.62 (28.16)	28.57 (31.16)
	0.3	0.6	0.00 (0.00)	0.00 (0.00)	14.81 (22.35)	24.81 (29.81)	27.38 (31.02)	33.33 (34.53)
EC-2	0.1	0.2	0.00 (0.00)	3.33 (6.14)	3.70 (6.49)	10.37 (15.34)	31.54 (33.60)	28.57 (32.30)
	0.3	0.6	6.67 (12.29)	3.33 (6.14)	14.81 (22.35)	13.70 (17.55)	30.95 (33.16)	33.33 (34.65)
Blank formulation			0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	3.33 (6.14)	4.17 (6.90)	9.52 (14.80)
Pongamia oil 1%			3.33 (6.14)	0.00 (0.00)	0.00 (0.00)	6.67 (8.85)	35.71 (36.53)	19.04 (25.56)
Neem oil garlic emulsion 2%			6.67 (12.29)	3.33 (6.14)	25.92 (30.49)	24.81 (29.81)	45.83 (42.57)	33.33 (35.16)
Thiamethoxam 25 WG 0.03%			33.33 (35.20)	38.15 (38.07)	62.96 (52.53)	81.48 (68.85)	100.00 (90.00)	100.00 (90.00)
SE(m)			3.828	3.868	3.729	7.064	4.904	5.644
CD (0.05)			(11.575)	(11.695)	(11.274)	(21.361)	(14.829)	(17.067)

CNSL- Cashew Nut Shell Liquid, EC-1 -20% CNSL, EC-2 – 20% CNSL + 20% pongamia oil, E- Effective dose AE - Above effective dose
*Corrected with Abbot's formula over control,
Mean of 10 adults per treatment with tree replication Figures in the parenthesis are angular transformed values, DAT- Days after treatment

Discussion

Safety of CNSL formulations were evaluated based on the phytotoxicity and toxicity to natural enemies and pollinators. The phytotoxicity of CNSL formulations at effective and double the effective doses were evaluated in the field at vegetative and reproductive phase wherein no phytotoxic symptoms were observed. Technical CNSL obtained from the cashew processing industry has a vesicant action due to the presence of cardol (Wassermann and Dawson, 1948) [11]. The dose for pest management in cowpea arrived at in the present study is 0.1 and 0.3%. Even at double the recommended dose of 0.6 percent no toxic symptoms were observed with respect to yellowing, scorching, epinasty and hyponasty on the crop. The slight necrosis observed at this dose was well within the safe limit of rate 1 in the 0 - 10 scale in the phytotoxicity proptocol of Central Board of Insecticides and Registration Committee (CIB&RC). Andayanie *et al*. (2019) [1] opined that the viscous extract of cashew nut shell up to 3% level did not caused phytotoxic symptoms in the leaves of soya bean, however 6% caused phytotoxic symptoms.

Toxicity of CNSL formulations at effective and double the effective doses were evaluated in the laboratory against predator and parasitoid using *Coccinella transversalis* and *Bracon* sp. At the effective doses no mortality of the coccinellids was observed at 1 DAT. At 5 DAT also the effective doses were found to be safe with mortality below 20%. The same trend was noticed with *Bracon* sp. also though the mortality at 5 DAT was around 30%. Mortality of predators and parasitoids treated with CNSL was significantly lower than those treated with chemical check and was on par with that of other commonly used botanical pesticides. Even at double effective doses the CNSL treatments did not produce mortality exceeding 35% ensuring the safety to natural enemies in comparison with chemical pesticides. Isman *et al*. (1992) [3] reported that neem based botanical pesticides have no detrimental effects on predatory coccinellids, chrysopids and syrphids. The safety of the neem products to the parasitoid *Telenomus principium* has been recorded by Klemm and Schmutterer (1993) [5].

Conclusion

CNSL based botanical formulation can be considered as a better option for organic pest management as it is proved to be safe to natural enemies without causing any phytotoxic symptoms both at the effective dose and above the effective dose. The phytotoxicity of CNSL formulations at effective (0.3%) and double the effective doses (0.6%) were evaluated in the field at vegetative and reproductive phase wherein no phytotoxic symptoms like yellowing scorching, necrosis, epinasty and hyponasty were observed on cowpea as per phytotoxicity rating scale of Central Insecticides Board and Registration Committee (CIB & RC). No significant adverse effect was observed against natural enemies, *C. transversalis* and *Bracon* sp for CNSL formulations when evaluated in the laboratory.

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