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Research Article
Effectiveness of Cashew Nut Shell Liquid (CNSL) with Bait Formulation Against *Iridomyrmex cordatus* as a Vector of Cocoa Pod Rot Disease Caused by *Phytophthora palmivora*

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Abstract
Background and Objective: Cashew Nut Shell Liquid (CNSL) is a biopesticide that is environment-friendly in controlling ants *Iridomyrmex cordatus* is a vector pot rot disease caused by *Phytophthora palmivora*. The research aimed to obtain the best concentration of cashew nut shell liquid formulated as smart food bait to tackle the ant population of *Iridomyrmex cordatus* due mainly to a vector of *Phytophthora* pod rot. **Materials and Methods:** The research was carried out in cocoa areas in South Sulawesi with a randomized block design consisting of six treatments. Feeding trials consisted of food bait formulation that was added with cashew husk, respectively 1, 10, 20% and 17.5 g carbaryl (recommended dose) and control (without cashew husk or carbaryl). Each trial was replicated five times and set near to petiole and ant tunnel nest. The research focused on the number of ant colonies established in the trees until food bait was given. **Results:** The trial with CNSL concentration had a positive effect on the ant population the higher concentration given, the lower the ant population was obtained. Meanwhile, the population of *I. cordatus* had a positive contribution to the increase of disease incidence of *Phytophthora* pod rot disease. Feeding trials with 1 and 5% CNSL concentrations were less effective to limit ant population and disease incidence according to the efficacy test. In contrast, the trials of 10 and 20% were able to control population density and disease incidence due to over 51.39% efficacy test. **Conclusion:** There was a positive trend of CNSL concentration to reduce the ant population of *I. cordatus*. There was the greatest association between the increase of ant population density and the increase in disease incidence of *Phytophthora* pod rot.

Key words: Feeding trials, food bait, CNSL, *Iridomyrmex cordatus*, *Phytophthora* pod rot

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Iridomyrmex cordatus, which is one of the ant trees, has a considerable influence on food webs in the agricultural ecosystem. The presence of *I. cordatus* in the number of valuable agricultural commodities raises a controversy. As a successful predator or scavenger, on one hand, the ant has a powerful and successful species in hunting and praying numerous animals and insects which are associated with crops and with its real-time protection services in the field the farmers take advantages[1,2]. On the other hand, the existence of the ant population in commercial commodities such as cocoa raises numerous issues. One of the problematic impacts is as the agent of *Phytophthora* pod rot disease[2]. Ant *I. cordatus* contributed to fungal spread from crop to crop, in spreading the pathogen to cocoa pods[3,4].

Phytophthora pod rot disease caused by *Phytophthora palmivora* is one of the most dangerous global cocoa diseases and in Sulawesi, the disease is more rampant with a much greater impact than any kinds of biological cocoa pests and diseases[5-8]. The wet season is one of the main causes behind the disease prevalence since the range of ripening time and peak harvest is almost rainy season[9,10]. A wetter field triggers the cause of pod rot disease, *P. palmivora*, conveniently infecting the tree and pods[8,11]. The disease incidence in the wet season is much higher than dry season[10]. Fungi of *P. palmivora* is a typical s-born pathogen that originally occupies the rhizosphere and it can form chlamydospore once the extreme condition is faced[11]. A group of insects is a potential agent to spread the pathogen such as termites and ant[12]. Ant species of *I. cordatus* has convincingly evidenced to associate with pod rot disease and its pathogen in Sulawesi[9]. The disease development caused by humidity level and a group of insects underpinning the infection process of the pathogen using microbial decomposer needs to be considered for cocoa pathogens as well[13].

A preliminary study was undertaken to show specific lesion occurrence once the body of *I. cordatus* was attached to a fresh pod[2,4]. The main reason behind of effectiveness of *Phytophthora* pod rot disease spread was due to tunnel nest of ant built from organic matters which is contaminated by spores, connecting niche and pathogen and providing inoculum sources of *Phytophthora* pod rot disease[8,11]. The pathogen infects all stages of cocoa pod development and if attacked immature pod leading to Cherelle wilt[11,14].

Excessive tunnel nests built in the tree and the presence of active worker ant population during foraging activity lead to an effective controlled method with pesticide apply and therefore alternative method such as artificial poisonous food bait of ant was proposed to control agent of disease spread[15]. The principle of the method was that the colony of ant workers was expected to carry lethal bait to the main nest and then killed the colonies and the queen[3]. It was evidenced that the population of *I. cordatus* has greatly contributed to the increase of disease incidence of pod rot disease[4]. Once 200 colonies of ant reached to occupy cocoa tree, 9.3% of *Phytophthora* pod rot disease increased per week[2]. The association between *I. cordatus* and disease and cashew is still unknown and therefore, this paper will reveal the significant role of the cashew tree in plant protection and environmentally friendly. Cashew (*Anacardium occidentale*) generates a shell consisting of outer endocarp, mesocarp and inner epicarp layers. The only inner epicarp layer like testa is necessary for the nut industry since it produces nuts while cashew husk (endocarp and mesocarp layer) is generally seen as useless material value as agricultural waste[15]. Shell has mesocarp with tremendous benefits for agricultural products such as natural pesticide[15-18]. Like the spread of *Phytophthora* pod rot disease in cocoa plantations, this activity was focused on testing the most effective concentration to control the population of disease vector ants on cocoa trees.

MATERIALS AND METHODS

Study area: The application of Cashew Nut Shell Liquid (CNSL) with bait formulation against *Iridomyrmex cordatus* was conducted from March to November, 2021 in the cocoa plantation located in Luwu Regency, in the province of South Sulawesi, Indonesia.

Feeding trials for the ant population: Food baits for ant colonies consisted of shrimp paste. To make shrimp paste, the composition of the mixture consisted of 0.25 (50 g shrimp flour):1.0 (100 g coconut sugar): 0.5 (200 g flour). The mixture was added $\pm$10 mL condensed milk as a stimulator and active ingredient for anti-microorganism. Subsequently, all mixtures are poured into one medium and shaped like a paste. The paste was separated and respectively added 1% of CNSL (346.5 g paste+3.5 g cashew husk flour), 5% of CNSL (332.5 g paste+17.5 g cashew husk flour), 10% of CNSL (315 g paste+35 g cashew husk flour) and 20% of CNSL (280 g paste+70 g cashew husk flour). Those paste mixtures were pelleted and incubated at 40°C for approximately 30 min. However, before incubating the pellet, the paste made was with a somewhat soft texture.

Trial design and efficacy test: The trials were set with a randomized block design which was placed into about

Table 1: Score of pod damage

Scores	Damage levels	Pod lesion (%)
0	No lesion	0
1	Pod lesion	>0-25
2	Pod lesion	>25-50
3	Pod lesion	>50-75
4	Pod lesion	>75

900 trees of cocoa surrounding with 1 ha area. The tree was grouped into 3 plots for every trial with five replications. Testing effective food bait made from a different concentration of CNSL and without CNSL and carbaryl (control) as a comparison was conducted. Observation of ant species was commenced once food bait was formulated following the method of Gassa *et al.*[2,3,19]. Food bait mixture separately consisted of CNSL in different concentration levels (1, 5, 10 and 20%), 17.5 g carbaryl (recommended dose) and control (without CNSL and carbaryl), respectively. Every mixture was tested in the field and the trials were set to the tree with 5 replications and mixed. This study focused on ant population density and the development of tunnel nests before and after trials. Also, the effect of the trials on disease incidence of *Phytophthora* pod rot was tested with ANOVA and *post hoc* test with a 5% Tukey's test.

The diversity of ant population was estimated with the following method of Khoo and Way in Gassa and Junaid[3] consists of 4 scores and categories such as, (1) Fewer (<50 ant population seen occupied in the branch, stem but no tunnel nest development), (2) Mild (Up to 200 ant population density and commencing development of tunnel nest), (3) Many (200-500 ant population density in the stem and branch and tunnel nest built) and (4) Abundant (>500 ant population density occupied the tree and numerous tunnel nest linkage built).

Testing the effect of feeding trials on disease incidence of *Phytophthora* pod rot: The observation of ant colony was undertaken after feeding trials in different concentrations including 1, 5, 10 and 20% CNSL, 17.5 g carbaryl (recommended dose) and control (without CNSL and carbaryl). Ant colony was weekly observed with a focus on the presence of ant colony and tunnel nest on the tree. The observation also was intended to investigate the association of ant colony and disease incidence in different level disease categories. Finally, *Phytophthora* pod rot disease was observed once ripe pods occurred. Mature pods were collected and separated from infected and healthy pods. The weight of healthy pods was counted to obtain the average bean weight estimation for every harvesting season. Disease incidence was categorized as follows in Table 1.

For disease incidence, the equation was counted as follows[20]:

$$I\ (\%) = \sum \frac{n \times v}{N \times V} \times 100$$

Where:

- I = Disease incidence (%)
- n = Number of the infected tree to every damage level (pod lesion)
- v = Score of every damage level
- N = Number of observed pods
- V = Highest scoring pod lesion

Efficacy test: Criteria of efficacy test were followed with a disease incidence of *Phytophthora* pod rot. The test of efficacy level aimed at the main trials with different CNSL concentrations as follows[20]:

$$TE\ (\%) = ISK\text{-}ISP\ (ISK)^{-1} \times 100$$

Where:

- TE = Efficacy level (%)
- ISK = Disease incidence of *Phytophthora* pod rot on the control (food bait without CNSL and carbaryl)
- ISP = Disease incidence of *Phytophthora* pod rot in the trials

The trials of food baits were effectively obtained from the test once the TE value represented at least 50% and had a significant sign to the control.

Data analysis: All trials were examined with a simple and descriptive quantitative and the effect of trials on ant population density and disease incidence was tested separately with ANOVA and Tukey's test.

RESULTS

Efficacy test of food bait formulated with CNSL: The efficacy of food bait trials formulated with CNSL and carbaryl in reducing ant population associated with the disease incidence in Luwu District was performed in Table 2. According to Table 2, the results demonstrated that after the second to the end observation, the trial of 20% CSNL (1.00) was shown to have the greatest consistent effect to control the density of the ant population. For the trial of 10%, CNSL was 1.75, the effect commenced to show in the fourth observation. It has been evidenced that the 20 and 10% CNSL concentration (1.00 and 1.75) was much fewer than in the trials of 1 and 5% concentration (3.50 and 2.75) of CNSL formulated into food bait.

Table 2: Performance of population of *Iridomyrmex cordatus* in different CNSL concentration levels

	Scoring average of *I. cordatus*/observation (weeks)										
Trials	1	2	3	4	5	6	7	8	9	10	11
CNSL 1%	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	3.50^{ab}
CNSL 5%	4.00^{a}	3.75^{ab}	3.75^{a}	3.75^{ab}	3.75^{a}	3.75^{a}	3.75^{a}	3.75^{a}	3.25^{ab}	3.00^{ab}	2.75^{bc}
CNSL 10%	3.75^{a}	3.50^{ab}	3.25^{ab}	3.25^{b}	2.75^{b}	2.75^{b}	2.50^{b}	2.25^{bc}	2.25^{c}	2.00^{bc}	1.75^{cd}
CNSL 20%	3.75^{a}	3.00^{b}	2.50^{b}	2.25^{c}	2.00^{c}	2.00^{c}	1.50^{c}	1.50^{c}	1.25^{d}	1.25^{c}	1.00^{d}
Carbaryl 17.5 g	3.75^{a}	3.50^{ab}	3.50^{a}	3.25^{b}	3.00^{b}	3.00^{b}	2.75^{b}	2.75^{b}	2.75^{bc}	2.5^{b}	2.25^{c}
Control	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}
BNJ value	0.799	0.889	0.93897	0.72732	0.899	0.68573	0.96977	0.86569	0.93897	1.02142	1.20613

Numbers in the same column followed by the same alphabets indicate insignificant according to the DMRT test (p>0.05)

Table 3: A simple regression analysis explained the association between the trials and the dynamic population of *Iridomyrmex cordatus* by the times

Observation (weeks)	Linear equation	r = correlation coefficient	Trend of Y value
1	Y = 4.0008-0.0146X	r = -0.3559	3.99
2	Y = 4.0179-0.0511X	r = -0.7835	3.92
3	Y = 4.2094-0.0777X	r = -0.8469	3.98
4	Y = 4.0958-0.0897X	r = -0.8862	3.73
5	Y = 4.1010-0.1043X	r = -0.8940	3.58
6	Y = 4.0704-0.1070X	r = -0.9277	3.43
7	Y = 4.1119-0.1336X	r = -0.9192	3.18
8	Y = 4.0806-0.1362X	r = -0.9123	2.99
9	Y = 3.9875-0.1441X	r = -0.9425	2.68
10	Y = 3.8875-0.1446X	r = -0.9173	2.43
11	Y = 3.6375-0.1456X	r = -0.8864	1.99

Trend of the Y value results from linear equation and observation time (X) indicating the higher value of ant colony, the higher disease incidence of *Phytophthora* pod rot disease

Table 4: Association between ant population of *Iridomyrmex cordatus* and pod rot disease incidence caused by *Phytophthora palmivora* regarding the trials of different CNSL concentrations and carbaryl

	Scoring average of disease incidence/observation (weeks)										
Trials	1	2	3	4	5	6	7	8	9	10	11
CNSL 1%	0.75^{a}	1.75^{a}	2.25^{a}	3.75^{a}	6.50^{ab}	9.50^{ab}	13.25^{ab}	14.25^{ab}	14.75^{ab}	15.75^{ab}	16.75^{ab}
CNSL 5%	1.50^{a}	2.25^{a}	2.75^{a}	4.00^{a}	5.25^{abc}	7.75^{bc}	11.00^{abc}	12.00^{bc}	12.25^{bc}	12.75^{b}	13.75^{b}
CNSL 10%	1.25^{a}	2.50^{a}	2.75^{a}	3.50^{a}	4.00^{bc}	5.75^{cd}	6.75^{cd}	7.50^{de}	8.00^{cd}	8.25^{c}	8.75^{c}
CNSL 20%	1.00^{a}	1.00^{a}	1.75^{a}	2.50^{a}	3.00^{c}	4.00^{d}	4.50^{d}	5.25^{e}	5.50^{e}	5.75^{c}	6.00^{c}
Carbaryl 17.5 g	1.00^{a}	2.00^{a}	3.00^{a}	3.75^{a}	5.25^{abc}	7.00^{bcd}	8.25^{bcd}	10.75^{cd}	11.00^{bc}	12.50^{b}	13.25^{b}
Control	0.75^{a}	1.25^{a}	2.25^{a}	4.50^{a}	7.50^{a}	11.50^{a}	15.50^{a}	17.00^{a}	17.50^{a}	17.50^{a}	18.00^{a}
BNJ Value	1.88964	2.20542	3.17728	2.91938	3.0666	3.17959	4.14816	4.44402	4.86697	4.17816	3.91676

Numbers in the same column followed by the same alphabet indicate insignificant according to the DMRT test (p>0.05)

Statistically, a simple linear regression analysis provides the solution Y = 3.6375-0.1456X with correlation coefficient was r = -0.8864 in Table 3. The demonstrates an association between the trials of CNSL concentration and the dynamic ant population of *I. cordatus*. The finding suggested that there is a positive trend obtained from the effect of level concentration of CNSL (X) on the dynamic population of ant (Y).

The relationship between the *Iridomyrmex cordatus* ant population and the incidence of *Phytophthora* pod rot disease: The association between the ant population and cocoa disease incidence is described in Table 4. Table 4 shows the trials of 20% CNSL had a scoring average of disease incidence was 6.00. Statistical test results showed a real difference in trials of carbaryl 17.5 g and control of 13.25 and 18.00, respectively. The association between the dynamic ant population of *I. cordatus* and pod rot disease incidence caused by *P. palmivora* show that the linear equation of Y = 2.4931+3.8681X with correlation coefficient was r = 0.9220 in Table 5.

The results demonstrated that from the first to fourth observation, almost all trials were shown to have a similar effect to the control but after the 5th week observation of 20% CNSL (3.00), the significant sign of almost all trials was in contrast to the control was 7.50. The trials of 10 and 20% CNSL concentrations were 8.75 and 6.00 demonstrated to have much more consistency decreasing *Phytophthora* pod rot disease than any other trials. The highest incidence of disease in controls (18.00) was followed by a trial of 1% CNSL concentration (16.75).

Table 5: A simple regression analysis explains the association between dynamic ant population by the times and pod rot disease incidence caused by *Phytophthora palmivora*

Observation (weeks)	Linear equation	r = correlation coefficient	Trend of Y value
1	Y = 0.8316+0.056X	r = 0.0191	0.89
2	Y = 0.7473+0.2747X	r = 0.1076	1.29
3	Y = 0.6+0.5X	r = 0.2299	2.10
4	Y = 0.7672+0.8356X	r = 0.4999	4.12
5	Y = -0.5636+1.7354X	r = 0.7414	8.12
6	Y = -1.7816+2.8732X	r = 0.8349	15.44
7	Y = -1.4456+3.7605X	r = 0.8768	24.88
8	Y = -0.7939+3.8529X	r = 0.9101	30.01
9	Y = 0.3707+3.7896X	r = 0.8973	34.39
10	Y = 1.0169+3.8362X	r = 0.9329	39.01
11	Y = 2.4931+3.8681X	r = 0.9220	44.95

Trend Y value results from linear equation and observation time (X) indicating the higher value of ant colony, higher disease incidence of *Phytophthora* pod rot disease

Table 6: Efficacy test level of cashew husk (CNSL) toward *Iridomyrmex cordatus* and disease incidence of *Phytophthora* pod rot disease

Trials	Efficacy test level (%)
Control	0.00*
CNSL 1%	6.94*
CNSL 5%	23.61*
CNSL 10%	51.39
CNSL 20%	66.67
Carbaryl 17.5 g	26.39*

*Ineffective level efficacy of trials if fewer than 50% efficacy test level, results for the efficacy test level of trials CNSL 20% and CNSL 10% showed values were 66.67 and 51.39%, which were higher levels than others

Efficacy test level of cashew husk (CNSL): In terms of efficacy test of food bait formulated with CNSL consisting of anacardate acid towards *I. cordatus* as a vector for *Phytophthora* pod rot disease was shown in Table 6. Results for the efficacy test level of trials 20 and 10% CNSL showed values were 66.67 and 51.39%, which were higher levels than others. The trials performed of 10 and 20% CNSL over 50% efficacy test level which is an effective concentration to limit ant colony in the field. Meanwhile, 1 and 5% CNSL, 17.5 g carbaryl and control are alike to show less effectiveness for ant colony limitation according to statistical efficacy test which is shown to have a much fewer level.

DISCUSSION

The results demonstrated to have the significant sign of CNSL trials (food bait with CNSL formulation) especially 10% and 20% concentration of CNSL against ant population and cocoa pod disease incidence. The present experimental results of Paiva *et al.*[16] and Dourado *et al.*[21] show that were obtained with increased concentration of phytopesticide cashew nut shell liquid with the decreased time of exposure, to bring about 50% mortality of insects. A successfulness to control the agent and the cause of *Phytophthora* pod rot disease is because of robust phenolic compounds contained in the husk of cashew[18,20]. In general, Cashew Nut Shell Liquid (CNSL) contains 23-34% oil[15]. Cashew liquid is one of many sources of robust natural phenolic compounds and has a potential natural pesticide to be developed[18]. Saenab *et al.*[22], Morais *et al.*[18] and Gassa and Junaid[3] suggested that due mainly to 80% anacardate acid, CNSL can be used as an insect repellent. Not just insect repellent role, antibacterial and antifungal are other roles of the substances of CNSL since it consists of anacardate acid aiming to break the pathway of *Prostaglandin synthetase*[18]. This enzyme has a vital role in prostaglandin development to support the physiological and reproductive systems of insects[23].

Likewise, the trial of 17.5 g carbaryl also performed a significant sign with less ant population than the concentration of 1% of CNSL and the control. The CNSL extract affects the controlling activities of insects an effect that had been reported by many authors such as *Callosobruchus maculatus*[23], 3rd-stage of *Aedes aegypti* larvae[21] and 3rd-stage and 4th-stage of *A. aegypti* larvae[16]. The scoring average of ant population density was about 2.25 but it seemed to have a much higher than trials of 10 and 20% concentration. In this trial, the ant population was found to have a somewhat density of about 200 ants population density by commencing the development of the tunnel nest. The finding indicated that the trial of carbaryl seemed to have less effectiveness to reduce the ant population. It is assumed that the strong smelly odour may lead to the repellent of the colonies.

The cause of *Phytophthora* pod rot disease can survive in the rhizosphere and mixed with organic matters so that with foraging activity of ant colony the inoculum is accidentally taken leading to the risk of a high infectious cycle. Ndoumbe-Nkeng *et al.*[24] stated that root and organic matters are the primary infective inoculum when initial rain occurs and then infected pods on the canopy are the seconder inoculum which is directly impacted by the loss of yield. It is statistically seen that there is the greatest correlation between foraging activity to carrying pathogen of *Phytophthora* pod rot disease. The score of ant colony tends to increase to the maximum population while disease incidence of *Phytophthora* pod rot is subsequently high by the times of observation from 0.05 to 3.86 with near 1.00 values of coefficient correlation.

CONCLUSION

There was a positive trend of CNSL concentration to reduce the ant population of *Iridomyrmex cordatus*. The 10 and 20% concentrations had a significant sign of limiting ant population until the fewer score of *I. cordatus* was established. There was the greatest association between the increase of ant population density and the increase in disease incidence of *Phytophthora* pod rot. Due to testing efficacy of fewer than 50%, feeding trials of 1 and 5% CNSL concentration and 17.5 g carbaryl were ineffective to limit disease incidence. In contrast, feeding trials of 10 and 20% concentration were shown to have over 50% of efficacy test.

SIGNIFICANCE STATEMENT

This study has significant signs of CNSL trials (food bait with CNSL formulation), especially with 10 and 20% concentration of CNSL against ant population and cocoa pod disease incidence. A successfulness to control the vector agent and the cause of *Phytophthora* pod rot disease is because of robust phenolic compounds contained in the husk of cashew.

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