



Phytomyrmex cordatus

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## Phytophthora black pod disease of cocoa caused by *Phytophthora palmivora*: Development of bio-fungicidal package in controlling the disease and the vector by food bait

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### ABSTRACT

The aim of study was to achieve a ready natural fungicide formulated in use as an effective environmental technological package. The formulation consisted of suspension and pellet due mainly to familiar usage by farmers in controlling particularly Phytophthora black pod disease (PBPD). Natural concept to control spread disease by ant vector was purposed. In fact, it was reported that up to 90% loss of yield in wet season in Sulawesi that is main cocoa production and population. Likely wet season, in dry season, annual significant loss was led by ant as vector. There were three of trials including; firstly, testing citronella (seraiwangi extracted) and cashew nut shell liquid (CNSL) made from cashew husk extracted was purposed to limit lesion disease development in pod surface; secondly, in the field, testing effectiveness in both plant extracted against the pathogen and; thirdly, testing plant extract mixed with food bait for ant as disease vector. The disease incidence was measured by scale level. Disease categorized from 0 (healthy pod), 1 (up to 25% pod lesion), 2 (25-50% lesion), 3(50-75% lesion) to 4 (more than 75% lesion). Disease incidence was measured as following:  $I = \frac{\sum(U \times V)}{Z \times N} \times 100\%$ ; I (disease incidence), U (total of tree infected in each disease category), V (score in each pod lesion), Z (high score). The result, in the lab activity, showed that both 5% and 10% CNSL concentration was greater limit of PBPD development than 5% citronella concentration, both NSCL and citronella treatments showed the lowest disease incidence (only scale 0.25 ~ 6.25% pod damage), and followed by 10% concentration was in score 2.75 ~68.75% of pod damage respectively. By ant control, lower ant population investigated (scale 1.5 or 50 to 200 ant colony) was in 20% CNSL concentration than other trials.

**Keywords:** bio-fungicide, formulation, Phytophthora black pod cocoa disease, *Iridomyrmex cordatus*

### INTRODUCTION

Sulawesi cocoa plays a very important role in development of national cocoa industry. It was evidenced that majority of cocoa population (65%), production (70%) and farmers (53%) are from the region [19]. As a high attention of government in the region, it was created the cocoa regulation into national economy. The Central Government has only laid Sulawesi region over focusing on national cocoa commodity where Sulawesi is the fourth of 6 (six) national economic corridors [16].

Compare to other world cocoa producer countries, the Sulawesi farmers tend to gain more about 80% of world cocoa but the price incentive obtained has not been maximized due to production and productivity issues [7]. The farmers are not able to provide beans in large number in local market. In 2008, production of Sulawesi cocoa reported was about 501 thousand tonnes of total and productivity was about 500 kg<sup>-1</sup> ha [19]. Since last decade, national cocoa production has been continuing to fall. Most recently, national cocoa production estimated hit about 300 thousand tonnes [12]. Many obstacles have been facing the increase of cocoa productivity such as lack of good

agricultural practices, ageing tree population, and intensive pest and disease infestation and recently issue is ageing cocoa farmer population [10]. In cocoa disease alone, a serious cocoa disease significantly reducing annually bean quantity and quantity due mainly to damaging pods and killing trees is *Phytophthora* black pod disease (PBPD) and stem cancer disease caused by *Phytophthora palmivora*. Yet, the controlling disease (pathogen) has sometimes been ineffective due mainly to a relatively less farmers' attention and rapid spread disease helped by agents and wider habitat (soil and material decomposed manner) in cocoa farm Sulawesi. *P. palmivora*, causes PBPD, has a wide host range and reduces a significant global cocoa production, up to 30% yield loss [8] and about 10% trees killed annually [11]. In major national cocoa population (about 67% of total), Sulawesi cocoa suffered from the disease in wet season that has damaged up to 90% yield loss and reported up to 60% economic loss per annum [22].

A wide spread of zoospore (inoculum source) in nature is helped by water splash, wind, insects, contaminated organic matters on the ground. Soil is a very crucial factor in developing the disease cycles particular wet season as it is a medium where inoculum source of pathogen is favorable to grow. Subsequently, a very essential determination of the disease development in the field is the presence of infective propagule pathogen released such as motile zoospore. In spread disease by water splash, once propagule released from infected pods, it then falls and is propelled by wind among canopies to meet a healthy pod and then a new infection commences. Understanding cocoa disease cycle caused by *Phytophthora* spp. in the ground was investigated by [21] in Bahia Brazil. The findings were that once sporangia from root infection release zoospores in the ground water, they then move into the surface before carried by splash nearby pods for ready to begin a new infection in soil above. Subsequently, regarding the spread disease by water and rain, the disease significance was caused by rain wash reaching up to 30 infected pods throughout 5 month observation compared to splash and soil carriers.

A part from the spread disease by water, another known disease distribution is by insect such as ant that determines disease cycles as well. The ant was evidenced to carry sporangia of the disease (trees investigated faced high disease incidence) by foragers and in nest tunnel built in cocoa farm [13]. The soil and plant materials decomposed mixed with propagule were carried by foragers and workers. Furthermore, there was a positive correlation between the number and ant species foraging in the cocoa trees and the increase of disease incidence in the field that the study undertaken was in wet season. Regarding the weather, more severe disease incur when high humidity occur with the manifestation of ant colonies in active around cocoa trees [23].

Diversity of pathogen species in the field has threatened yield reduction as the other issue. Evolutionary crop pathogen occurs in environment that generates virulent alleles within populations as a result of genetic variation within pathogen populations, [17] can limit yield production. Therefore it needs strategies in controlling PBPD pathogen [5]. Focusing on control of PBPD, efforts were just specifically to use synthetic chemical approaches that may have a potential harmful for agricultural ecosystem. To some extent, it can be practically denied, the farmers are happy to use synthetic chemical control because classical reasons are offered such as an ease in utilization and rapid to see its impacts. However, long term impact to living organism in ecosystem and land as well as aware of farmers and costs should be considerably accepted.

There are many harmful fungicides driven by synthetic substances investigated in cocoa farm such as cupric, Mancozeb, Copper Sandoz, Cupravit, Vitigran Blue, Cobox and Nordox 56 WP. However, the formulations, in contrast in general expectation, are unlikely to meet effectiveness in controlling PBPD pathogen. Despite the fact that easy to use, the hazardous chemical formulated in long term use might not benefit for farmers due mainly to agricultural input expenses, safety reasons and new issues such as raising a new resistant genotype impacting to controlled difficulty (cost). Seeking attractive, friendly and environmental options to control is becoming an imperative consideration.

The trend of fungicide, plant extracts-driven to tackle PBPD in the farm is still an inadequate, in particular ready fungicide use, even though the efforts to control the diseases were successfully undertaken in Lab scale. Potential utilization of plant extract as promisingly bio-control generally known is lemongrass (binomial name: *Andropogon nardus*, local name seraiwangi); graviola (binomial name: *Annona muricata* var. *subonica*, soursop fruit, local name sirsak); maja fruit (Binomial name: *Aegle marmelos* Correa, local name Maja); cashew (binomial name: *Anacardium occidentale* L. cashew, local name mente). In the lab trial for examples, [20] assessed that testing 750 ppm concentration seraiwangi into disk can limit to 76% of growth diameter of *P. palmivora* colony and to 83% its biomass. [1] emphasized, giving 0.1 mL volatile compound and 0.075 mL citronella fraction from serewangi extract per petri-disc are capable of killing filamentous fungus (ceasing 100%). Another potential natural product to be developed is mente waste (husk).

In spite of managing waste of husk especial in harvesting season, abundant husks are separated from fruit and nuts are unfortunately wasted. The only fruit and nuts benefit according to the farmers. Apart from economic fruit and

nut values, the husk is promisingly in use as bio-control due to containing cashew nut shell liquid (CNSL). Finding CNSL compounds in cashew wasted is not difficult way and it can be conventional technique or using chemical extraction. [14] tested chemical compounds in husk cashew and found up to 30% CNSL. It was broadly known that chemical compound in cashew husk (CNSL) consists of 70% *anacardat acid* and 25% *cardol*. Whilst, other chemical compounds, *cardanol* and *methylcardol* remaining are antimicrobial substances [24]. Both *cardol* and *cardanol* substances are derivate from *tannin*, the poisonous natural polyphenol. [25] investigated for natural polyphenols from tannin, producing antimicrobial effects due to unspecified mechanism to protein of living microorganism and cell membrane microbial destruction as well as denaturation of bacterial protein. *Cardol* and *anacardat acid* benefit as additive substances for antiseptic purposes. *Cardanol* itself is used as making pesticide formulation and drug materials after hydrogenated process [9].

Therefore, the study on both seraiwangi (lemongrass, *A. nardus*) and husk of cashew (mente, *A. occidentale*) were undertaken to see potential use and development of biocontrol PBPD in massive scale. For ant population control due to as a vector of PBPD, Sevin 85 S (insecticide) was sprayed to pod surface infected. The use of Sevin 85 S was purposed to reduce its population number in the trees regarding spread PBPD [2]. In direct spread PBPD pathogen to cocoa surface by ant, inoculum source can nudge in the body and be carried to nest tunnel reaching up to pod surface [23]. The initially pod infection process begins at pathway. Therefore, because *I. cordatus* important agent causing the disease of PBPD, the need for alternative tactic to control is urgently to be undertaken. It is hypothesized that a bait concept mixed with poisonous food (it is believed rare pest control tactic used) maybe an effective solution for ant restriction to trees and spread PBPD. Due mainly to activity of ant inside the tunnel fully covered by soil and organic matters, using such a contact insecticide is likely in effective technique. The food bait, regarding preference tactic, then mixed with toxic chemical substance is believed as a smart concept to control due to based on foraging habit of ant. This tactic can kill the disease agent as workers and foragers by being expected them to carry the poisonous food bait to their nest and to feed their queen and new generations [4].

The utilization of active ingredients made from plant extracts to control spread PBPD through ant activity occupying cocoa tree is purposed in the recent study. It is expected that plant extract fermented is promisingly as bio control against pathogen of PBPD and its vector by using spray of pod surface, soil, feed bait to ant and nest tunnel. The result is expected to a role of reduce the inoculum source of disease in the farm.

## EXPERIMENTAL SECTION

The study was undertaken in the Lab of Plant Protection Department Faculty of Agriculture University of Hasanuddin and cocoa observation in Soppeng and Bone districts South Sulawesi for November 2015. The study conducted was divided into three sub-trials; the Laboratory and field assessments. On the lab trial, firstly, the assessment of citronella (seraiwangi, *A. nardus*) and cashew husk (mente, *A. occidentale*) extracted to control *P. palmivora* growth on the pod surface. Secondly, in the field assessment, it aimed to evaluate the ability of both seraiwangi and cashew husk extracted (CNSL) to decrease growth of black pod pathogen mycelium on pod surface. And thirdly, to test both products of plant extraction combined with food bait for purposing the PBPD vector.

### Extraction technique

Similar to cashew husk extraction technique, in seraiwangi extraction, 500 g matured leaves harvested and 25 g pulp (mature fruit) of maja (*A. marmelos* L.) were mixed. All materials dissolved, in order to obtain 1000 mL, were then added Methanol. To separate between suspension and waste, screen was used achieving crude suspension. 50% crude solution obtained was added 10 mL *teepol* as adhesive substance for lab and field trial purposes, the adhesive material is safe for crude extract suspension. Separately, leaves of soursop (local name: sirsak) and nimba fermented were grounded to 500 g and loaded 25 g of extracted maja prepared. Furthermore, in order to rapid activate plant chemical compounds into suspension, adding 1000 mL water of rice grains wasted and sufficient molasses (trigger of fast fermented process) were done and poured into a ready cumber. The supernatant was separated from suspension and waste material and supernatant harvested was gently stirred to be rather brown color. Brown supernatant harvested was transferred into the air-proof container for 14 days- fermentation. Product extraction was obtained and poured into the bottle for next assessment.

### Testing extract of seraiwangi in vitro to restrict diameter of *P. palmivora* hyphae

plant extract trial formulated to suspend was achieved by mixing and homogenizing all materials of seraiwangi suspension following 0% (none of extraction), 1%, 5% and 10% respectively were applied to whole healthy pod surfaces (about 10 cm pod size). In the field trial, the study was designed by using randomly completed design with 4 (four) replications (one healthy pod treated per replication). The total, therefore, in the field trial were 32 healthy pods.

**Testing extracts of seraiwangi and cashew husk against *P. palmivora* in the field**

In the field trial, preliminary observation prior to ensuring number of sampling, all trees were marked. Field assessment was designed with randomly split design consisting of 4 group observation. In each group there were 3 treatments, seraiwangi extracted (citronella) combined with cashew husk extracted (CNSL). Each treatment was repeated 5 times and every replication consisted of 5 trees. Therefore, the total of samples was 60 trees. 2 pods chosen in every trial were used and were marked.

Application of seraiwangi (citronella) and cashew husk (CNSL) extracted to pods (more or less 10 cm pod size chosen) were chosen surrounded by pod infected PBPD pathogen. 1%, 5% and 10% concentration were sprayed in healthy pod surface. A weekly application was only conducted in the morning, in order to avoid sunlight exposure, with 6-times frequent application. Prior to established trials, initial investigation on pod surface was done and it then was indicated by first observation. Subsequently, disease incidence was measured by scoring following;

**Score level of Pod damage (%)**

0: healthy pod

1: disease incidence > 0 – 25,

2: disease incidence > 25 – 50,

3: disease incidence > 50 – 75,

4: disease incidence > 75

There is disease incidence pattern following by

$$I = \frac{\sum(U \times V)}{(Z \times N)} \times 100\%$$

Note :

I = Disease incidence

U = number of trees infected based on level of pod damage

V = score value to every level of pod damage

Z = highest score value

N = Total pods observed

**Testing plant extracted fermented mixed with food bait for disease vector**

Prior to testing disease carrier, food bait preparation formulated [3 ; 4] were mixed with fermented sirsak and nimba extracts in different concentration level including 10%, 20%, 30%, 40% and 50% respectively and testing cashew husk extracts fermented by concentration of 1%, 5% 10% and 20% respectively. The bait treated were laid into pod surface and repeated 4 times. The objective was to investigate ant colonies (the disease vector) foraging around the bait. To see the effect of trials against PBPD pathogen, non-covariant analysis was undertaken followed by Tukey's test. The number of foragers visiting pod trials was accounted by following [15] technique (Table 1).

Table 1. Categorizing ant colony based on (Khoo & Way, 1991)

score	population	Number of ant observed on the tree
1	Light	Lesser than 50 colonies seen in branch and stem. None of nest tunnel built
2	Moderate	from 50 to 200 ant colonies observed and initial nest built
3	Many	from 200 to 500 ant colonies seen in branch and stem and nest developed
4	Abundance	Over 500 ant colonies seen and colonized the tree

Testing trials on mass scale would be conducted in cocoa farm incurring severe PBPD chosen in the next project.

**RESULTS AND DISCUSSION****Testing plant extracts, seraiwangi (citronella) and husk cashew (CNSL), in vitro to control the growth of hyphae**

Plant extracts obtained from seraiwangi and husk of cashew had an inhibitor effect of developing disease symptom that the effect varied based on concentration. It was evidenced that these treatments were capable of inhabiting symptom development. It was seen in cashew husk extract that the greatest concentration impacting to reduce symptom development was in 5% and 10%, followed by seraiwangi extract similar concentration to cashew husk. Comparing to however the control, all treatments affected to reduce the disease incidence (Table 2).

**Table 2. Diameter of lesion effused in pod surface caused by *P. palmivora* after spray of citronella and CNSL in different concentration**

Plant extract	Concentration (%)	Diameter of colony(mm)
Citronella (seraiwangi)	1	48.1 <sup>b</sup>
	5	39.2 <sup>c</sup>
	10	37.7 <sup>c</sup>
CNSL (mente)	1	52.6 <sup>b</sup>
	5	35.6 <sup>c</sup>
	10	33.6 <sup>c</sup>
None of extract (control)	0	64.6 <sup>a</sup>

Note: number in the same column followed by same words is non-significant difference on 5% level of Tukey's test.

By in vitro test, optimal inhibitors from serawangi and cashew husk extracted showed that using 10% concentration of serawangi was greater restriction to 37.7 mm (equal to 3.77 cm) disease development and followed by 5% in cashew husk was 33.6 mm (3.36 cm). It is assumed that those plant extracts are potentially to be developed as friendly environmentally pesticide formulation. If looking at the biochemical compounds in serawangi extracted, there are *citronellal* and *linalool* as well as  $\alpha$  *pinen*,  $\beta$  *pinen* and *methone* indicating as the greater anti-fungi. *Geraniol*, *citral* and *terpene* contained in this plant extract moderately hamper [18]. By concentration of 75 ppm, citronella (seraiwangi extracted) is capable of producing inhibitor about 76% of PBPD development and about 83% of mass filamentous pathogen was declined. However, once its concentration was increased to 1,000 ppm, fraction of citronella substance killed pathogen mycelium. Furthermore, another fraction compound from serawangi, volatile with 0.1 mL dosage combined with 0.075 mL per disc of citronella are capable of limiting 100% of mycelium or nothing hyphae diameter is investigated [20]. [1] stated that citronella can limit to 71.2% of *F. oxysporum* f. sp. *lycopersici*.

By testing plant extract made from cashew husk, it has a promisingly biocontrol due mainly to chemical compound as anti-microbes.[9] pointed out that *cardol* and *anacardat acid* benefit as antiseptic compound and when was hydrogenated, *cardol* can be utilized as drug and chemical formulation. The husk of cashew mainly contains of CNSL namely Cashew Nut Shell Liquid including 70% *anacardat acid*, 20-25% *cardol* which benefit for insecticide, bactericide and fungicide [14]. Small amount of *cardol* and *cardolmetil* fraction remaining still utilizes as antimicrobials [24].

#### Testing of CNSL and citronella in reducing PBPD pathogen in the field

Likely to in vitro test in the Lab, testing CNSL and CNSL in field against to disease incidence of PBPD caused by *P. palmivora* showed (Table 2) showed more significant reduce of pathogen than control as following;

**Table 3. The average of disease incidence caused by *P. palmivora* after spray of CNSL and citronella in different concentration**

Plant extract/ concentration (%)	Observation (week)/score										
	i	ii	iii	iv	v	vi	vii	viii	ix	x	
Citronella (seraiwangi)	1	0.5	0.75	0.75	2.0	2.5	2.5	2.5	2.5	2.75	3.25 <sup>b</sup>
	5	0.5	0.75	0.75	1.75	1.75	2.0	2.0	2.75	3.5	3.5 <sup>b</sup>
	10	0.5	0.75	1.25	2.0	2.0	2.25	2.75	2.75	2.75	2.75 <sup>c</sup>
CNSL (mente)	1	0.0	0.5	0.75	1.0	2.0	2.5	2.5	2.75	3.25	3.25 <sup>b</sup>
	5	0.0	0.0	0.0	0.25	0.25	0.25	0.25	0.25	0.25	0.25 <sup>d</sup>
	10	0.0	0.25	0.5	0.75	0.75	1.0	1.25	2.5	2.75	2.75 <sup>c</sup>
Control	0	1.0	1.0	0.75	2.75	2.75	2.75	3.0	3.25	3.75	4.0 <sup>a</sup>

Note: number in the same column followed by same words is non-significant difference on 5% level of Tukey's test.

#### Score Pod damage level (%)

0 healthy pod

1 range of lesion effused from 0 to 25,

2 range of lesion effused from 25 to 50,

3 range of lesion effused from 50 to 75,

4 whole pod surface covered by lesion

Regarding the average of disease incidence in the field by different CNSL and citronella concentration, it was stated that even though low level concentration (5%) was given, all trial had more considerable impact on reducing disease incidence than control. The lowest disease incidence of PBPD was in 5% CNSL concentration, 0.25 disease score, equaled to 6.25% pod damage, followed by 10% concentration of CNSL and citronella with 68.75% pod damage respectively. The control showed the greatest score of disease incidence during the study, posing 100% pod damage (mummification). The greatest impact of 5% CNSL concentration was caused *cardol* and *cardanol* compounds in CNSL oil are a group of natural polyphenols that poison to growth of *P. palmivora* hyphae. [14] pointed out that CNSL substance can be used as insecticide, bactericide and fungicides. The causes of toxicity in polyphenol group is

the impact of lysis on membrane cell and protein microbes by unspecific mechanism known as antimicrobials [25]. [9] emphasized that once *cardol* compound and *anacardat acid* are hydrogenated, it can be generated drug and formulation for pesticide. Similarly, CNSL produced by cashew husk extracted can be utilized as insecticide, bactericide and fungicide [14].

#### Testing plant extracts fermented in the field mixed with food bait for the disease vector

The trend of ant population score, *Iridomyrmex cordatus* as PBPD vector, in control (trial) increased and levelled of the end of study (Table 4).

Table 4. The average of ant population, *I. cordatus* as PBPD vector, after feeding bait mixed with fermented from sirsak (*A. muricata*) nimba (*A. indica* A. Juss) and mente husk (*A. occidentale*) producing CNSL

Plant extract/Concentration (%)		Observation (week)/ ant population									
		i	ii	iii	iv	v	vi	vii	viii	ix	x
Sirsak ( <i>A. muricata</i> )	20	3.75	3.75	3.75	3.0	3.0	3.0	3.0	3.0	3.0	3.0
	30	4.0	4.0	4.0	3.25	3.25	3.25	3.25	3.25	3.25	3.25
	40	3.75	3.75	3.75	2.75	2.75	2.75	2.75	2.75	2.75	2.75
	50	3.75	3.75	3.75	3.0	3.0	3.0	3.0	2.75	2.75	2.75
Nimba ( <i>A. indica</i> )	20	4.0	4.0	4.0	3.75	3.75	3.75	3.75	3.75	3.75	3.50
	30	4.0	4.0	4.0	3.75	3.75	3.75	3.75	3.75	3.75	3.50
	40	4.0	4.0	4.0	3.75	3.75	3.75	3.75	3.75	3.75	3.50
	50	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.50
Mente ( <i>A. occidentale</i> )	1	3.5	3.50	3.50	3.50	3.50	3.50	3.5	3.50	3.50	3.50
	5	3.75	3.75	3.75	3.75	3.75	3.75	3.5	3.50	3.50	3.50
	10	3.50	3.25	3.25	3.25	3.25	3.25	3.0	3.0	3.0	2.75
	20	2.75	2.75	2.75	2.75	2.50	2.50	2.0	2.0	1.75	1.50
Control	0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	3.75

The ant population tended to fall after four weeks observation in the extraction of sirsak and nimba fermented (Table 4). The average of ant population in these plant extracts was lower than in average of population in control. Meanwhile, in the trial of cashew husk (CNSL) in 20% concentration, the average relatively levelled of several weeks at the beginning but then the population was a significant fall from about 200 colony (in score 2) in particular the seventh observation to below 200 colony (score 1.5) in the end. In contrast, the highest ant population was seen in control with score 3.75 (more than 500 colony) throughout the study.

Furthermore, a slight effect in reducing ant population was seen at the trial of 40% concentration in sirsak. It is believed that seeds of sirsak (*A. muricata*) extracted and dissolved on the solution consists of about 45% fat, annonin and resin, that work for insects due to contact and stomach poisons. Annonin and resin substances also are used to control *Aphids fabae*, *Aedes aegyti* and *Brevicoryne brassicae* [6]. Insecticide derived from seeds of sirsak that can kill insect larvae and adults and also cease feeding activities of insect pests (antifeedants) as well as repellents. The natural substance in sirsak leaves can control orthopteran grasshoppers [14]. The natural substances from cashew husk, the CNSL was capable of pressing ant population to about 200 colonies. Cardanol substance in CNSL functions as insecticide and additive compound to formulate pesticide [9].

#### CONCLUSION

Testing symptomatic disease on pods with growing pathogen into specific media (V-8 media) and microscopic assessment showed that the cause of lesion effused was *P. palmivora* alone. In vitro test, CNSL obtained from cashew husk extraction was greater restriction of growing hyphae caused by PBPD in 5% and 10% concentration than citronella concentration (seraiwangi extracted). In terms of capable of pathogen hyphae restriction in the field, seraiwangi and cashew husk extracted trials showed that 5% CNSL concentration (cashew husk extracted) showed the lowest growth of hyphae (0.25 score equaled to 6.25% damaging pods), followed by 10% CNSL and citronella concentration impacting to 2.75 score of growing hyphae, similar to 68.75% pod damage. There was the impact of food bait trials against ant vector PBPD showed by 20% CNSL concentration, pressing to lower ant population in 1.50 score (equals to 50 – 200 colony) than other trials.

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