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TROPICAL YAM-TUBER (*DIOSCOREA HISPIDA* Dennst.): FORMULATION AND A FUTURE NATURAL RODENTICIDE TO CONTROL RAT PEST IN RICE FARMING

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ABSTRACT

Tropical yam, Dioscorea hispida Dennst, has bitter taste and rich chemical poisonous properties that may have a promising rat pest control. In this study, herbal rodenticide formulated with yam-tuber (locally named as Salapa; gadung; generally known as starch tuber). Dioscorea hispida was evaluated in terms of rat population control in rice farm. Finding an effective formulation would be massively disseminated on farm scale. The study was designed with completely random design consisting of three treatments such as 300 g, 600 g and 900 g of yam-tuber with three replications and a control. The results showed that mice behaviours fed baits were slow motion movement, quiet stay, shaking, paralysis before death. Tropical yam tuber formulated stimulates numbers of diet, body weight and mortality. The higher dosage was given, the more significant effect had. Males fed in average of baits; 2.35 g (300 g dosage), 1.94 g (600 dosage) and 1.81 g (900 g dosage) while females were 2.31 g, 2.00 g and 1.87 g bait. Body weight measurement in males were 4,16 g (300 g dosage), 3.54 g (600 dosage) and 3.26 g (900 dosage) while females were 4.73 g, 4.04 g and 2.77 g respectively. Mortality were initially found in the sixth, fourth and second day but 100% killed specimens were recorded in the twelfth (300 g dosage), the ninth (600 g dosage) and the fifth (900 g dosage) day.

Keywords – Tropical yam-tuber (Dioscorea hispida), rat pest, natural rodenticide, food bait

1. INTRODUCTION

One of major rice pests is rat species wherever it grows. Rat pest is alarmingly paid attention due to cause of significant damage to rice farm. By 2007 – 2011, total losses of rice production recorded in Indonesia was about 158,900 ha per year which 2,600 ha (rice mature) of total damaged[1] (Ditjenbun-Direktorat Perlindungan Perkebunan, 2013). Most recently, planting time on June 2016, one of rice production centre in South Sulawesi is Pinrang district has experienced a significantly unsuccessful yield (locally known as *puso*). A major cause is, after investigation, enormous rats devastated a wider range and large number of rice plantation which most of rice areas were rice mature[2] (Hakim, Rahim, Ibrahim, & Beddu, 2016). The farmers claimed that a wide range of devastating mature paddy only occurred sporadically overnight attack. instead, attack of rat pest partly remains amount of paddy damaged and can be recovery by giving fertilizer. Due to significant rice losses, high alarmingly attention is paid to design an effective rat pest control in the field.

Agronomic, physical and chemical controls are generally recommended but synthetic pesticide used often impacts more drawbacks than benefits. The use of pesticide in farms is undeniable to have high preference by rice farmers as it offers simplicity in use and knock down effect[3] (Priyambodo, 2003). The conflict of interest rises to find a solution of seeking an alternative control by using natural products based on farmer controlled habit. Using natural pesticide promotes less environmental issue, un-expensive technological package and non-resistance mechanism effect is a major reconciliation. Edible starchy tuber was identified to have varied robust chemical properties that may function to kill mammals like rat.

One of potential natural herbs to be developed in rice farming, due to having extreme toxic substances, in controlling mammalian rice pest is as group of dioscorean species. It has been believed that Dioscorean species has mode of action such as feeding stimulation disturbance and reproductive disorder and mating unconsciousness[3] (Priyambodo, 2003). In Dioscorean species, tropical yam-tuber (*Dioscorea hispida* Dennst.) has chemical properties as potential herb as bio-rodenticide. This yam based on function is grouped with fertility and mortality effects. *D. hispida* is known as a bitter yam, poisonous starch-tuber or neglected herb of dioscorea species (locally known as salapa; umbi gadung). Dioscorean species is the most popular yam in many tropical and subtropical countries both as pharmaceutical and staple food[4] (Han et al., 2014). This herb has poisonous alkaloid and steroid substances[5] (Kumoro & Hartati, 2015). *Dioscorea hispida* Dennst is a wild vegetation growing in rural areas and has a potential rat control[6] (Maspary, 2012). In Malaysia, *D. hispida* is used as anti-bacteria[7] (Azman et al., 2016). *D. hispida* has varied hazardous substances which is a wider known as solid alkaloids (e.g. dioscorin) that mainly causes paralysis effect when is intake to human and animals[8] (Kardinan, 2001). Alkaloid dioscorin has one or two nitrogen atom that is very often toxic effect. Another toxic substance is hydrogen cyanide (HCN). HCN bonds and shapes a glicoside cyanogenic that can devastate oxygen transportation to among layers. If oxygen transportation dysfunctions, breath disorder, paralyses and death are often unavoidable[9](Prichard, 2007).

A successful bait to attract and to kill rat in the field can be influenced by variation, appearance and additive substances given. An increase of rat appetite may be triggered by an attractive bait like colour and appropriate composition such as carbohydrate, protein and fat in equalized amount as well as texture, shape and size. The shape and type are necessarily to be considered in the field[3] (Priyambodo, 2003). For instance, segmented bait structure that was mixed 25% yam-tuber, rice, paraffin, caramel and additive substances is attractive to rat in the rice farm and effective to kill up to 70%[10] (Murjani, 2011). Therefore, the need for understanding rat response to lethal bait modified in order to avoid feeding deterrent was necessary.

2. MATERIALS AND METHODS

2.1 Materials

White mice as specimen were obtained in Department of Biology Faculty of Maths and Natural Science University of Hasanuddin. 3 month-mice were chosen and about 20-34 g weights of 24 males and 36 females were maintained in spacy cage compartments (50 x 20 x 20 cm^3). All mice were fed 300 g maize flour + 20 g dried shrimp + 20 g candlenut (kemiri: local name) + 20 g coconut + 20 g CMC as an initial adaptation to a new environment. All substances were mixed together and added sufficient water before being formed a block 2 x 1 cm^2. Regarding process adaption, the specimens also were provided water 50 mL.

2.2 Designing bait

In this experiment, using tuber of *D. hispida* (TDH) was mainly purposed. yam-tuber was washed and chopped before grounded by using kitchen mixer. In all handling process of making bait, gloves were worn. Additive substances given such as frown, candlenut (local name: kemiri; *Aleurites moluccanus*), dried coconut were grounded before being mixed to have dosage variation such as G0 (control) = 300 g yam-tuber grounded maize + 20 g (dried frown, candlenut, dried coconut and CMC); G_1 = 300 g TDH + G_0; G_2 600 g + G_0; G_3 900 g TDH G_0. All dosages were resembled to block 2 x 1 cm^2 which each block was 5 g weight.

2.3 Spacious trial on mice

The cage was shaped as a rectangle (100 x 20 x 20 cm^3) with 5 compartments. The wall and floor were made from thin wooden layer and front wall and ceiling was made of aluminium gauze. Every compartment was equipped with a plat and a water container.

2.4 Statistical Analysis

In Statistical analysis, the study was designed with complete randomized design consisting of four treatments and three replications in every treatment. One treatment consisted of five mice (2 males and 3 females). Initial stage was that all specimens were ensured body weights which the only one mouse was laid into every single compartment. Specimens were fed the block bait shaped and 50 mL drinking water given everyday. The observation for every specimen behaviour and mortality rate was daily undertaken until all specimens were killed. To determine body weight, number of bait remaining was assessed and calculated by digital measurement as following;

$$body\ weight = initial\ weight - current\ weight$$

while observation of specimen mortality was calculated by following;

$$percentage\ of\ killed\ specimen = \frac{\text{number specimen killed}}{\text{total specimen tested}}\ x\ 100\%.$$

Renewing baits fed in the same portion, adding drinking water and cleaning compartment from urine and wasted specimen were daily undertaken.

3. RESULTS AND DISCUSSION

3.1 Specimen behaviour after tested

Effect of treatments in the varied dosage given to behavior and mortality rate (Tabel 1) were clearly seen in particular day 2 to day 4. The specimens appeared slow motion movement, quiet stay, confusedness, shock, lack of senses and powerlessness. This finding suggested that severe behaviours occurred were apparently affected by dioscorin that sources from TDH. The dioscorin causes shaky and paralysis disorder and dioscin impacts to neuron disorder. Alcaloid dioscorin works to anticholinesterase which means cholinesterase is not able to break down transmitter of chemical ach (acethylcholine). Once acethylcholine accumulates in between synapsis system influencing an increase of amount transmitted stimulation, inhalation muscle continuously works impacting to paralysis and death.

Regarding recognising and intake bait given, entire baits were remained as specimen initially ate in small amount in order to recognise negative impact of bait. When bait intake has no toxic to the body, specimen enjoy and will finish entirely bait. Rat has a better inhalation system. Rat is able to allocate food, predator and rat species. Rat can recognise their trace family by using better inhalation system so that their territory is well known. Therefore, understanding inhalation behaviour is necessary to design typical poisonous bait needed whether it has a knock down effect or not. The need for harmless bait is very vital giving at the beginning test as specimen will familiarize to bait provided. Once specimen familiarizes with bait, the bait composition can then be added toxic substance until the bait is eaten to enrich a lethal dosage.

3.2 Testing feeding ability

Testing consumption level (in average) in both sexes showed that the male preferred trial G_0 (bait without yam-tuber) such as 2.51 g bait in average eaten. In contrast, trial G1, G2 and G3 were eaten amount of 2.35 g, 1.94 g and 1.81 g respectively. In females, 2.94 g bait eaten in the trial G_0 but they consumed amount of 2.31 g, 2.00 g and 1.87 g bait in the trial G_1, G_2 and G_3 respectively (Table 2). These finding indicated that G_0 trial was preferable due to non-poisonous food while giving yam-tuber made different in G_1, G_2 and G_3 trials. It is assumed that the need of food varies by rat to grow such as grains, grasses, insects, and juvenile fish. A daily need for food

for a young rat was about 10% of its bodyweight but wet food resources provided is up to 15% of its bodyweight need. For daily drinking water need, a young rat often requires from 15 mL to 30 mL. Carbohydrates as energy sources are essential need to grow and protein, mineral and lipid components are in balanced serving. If looking at composition of trials given, G_0 block trial consisted of flour of maize flour, frown, coconut, candlenut and CMC while G_1, G_2 and G_3 added Starch-tuber enriched daily need for food. Feeding TDH flour (trials) showed that specimens ate trials even small amount. Ideally, in nutritious balance, the need for carbohydrate is about 18%[11] (Sukarsa, 2010) and TDH chosen was due to rich of carbohydrate and TDH is one of staple food[12] (Bhandari & Kawabata, 2005) and medicine crop[4] (Han et al., 2014). TDH has rich for essential amino acid, vitamin C and mineral[13] (Nagai, N. Suzuki, Tanoue, Kai, & Nagashima, 2007). In this recently research, adding maize flour in the trial was expected to enrich energy and protein, similar to adding flour frown, coconut, and candlenut in the trials that consist of high protein, vitamin and mineral sources for specimens. Giving an average dosage of frown flour on the poisonous bait is preferable for mice[14] (Nababan, 2012). The trials of G_1, G_2 dan G_3 were non preferable for specimens due to dioscorin within yam-tuber and tannin (bitter taste).

3.3 Testing body weight after application

Testing body weight of specimen after application showed that the implication of TDH to the body weight in both sexes in every dosage (Picture 1) was significant. The average of body weight in dosage G_1 was 4.16 g (male) and 4.73 (female). For dosage G_2, body weight of male was 3.54 g and female 4.04 g. In dosage G_3, male was 3.26 and female 2.77 g. This indicated that the higher body weight in both sexes was in dosage G_1 but the lower was in the dosage G_3. These finding indicated that mode of action of starch-tuber impacted to antifeedant. In the G_3 trial, bodyweight of specimen was lower as chemical substances in the yam-tuber reduced bodyweight and killed specimen rapidly.

The decrease of body weight in the specimen tested suggested that a robust correlation between simultaneous loss of body weight and haemoglobin (blood glucose) levels of specimen indicated that was directly influenced by natural substances from starch-tuber. Yam-tuber affects haemoglobin dysfunction. Haemoglobin works as a red protein that is responsible for transporting oxygen in the blood of vertebrates and mammalian like group of rat. The finding is, that extract of starch-tuber has dosage 1260 mg/kg body weight that is able to decrease blood glucose in proportional amount within insulin. Once glucose is available in heart tissue, gluconeogenesis is active that may generate arise of producing glucose from other substrates such as protein decomposition. However, if gluconeogenesis is continually generated, glucose remaining is wasted through urination process. As a consequence, specimen body will be loss of weight[15] (Suriani, 2012). If looking at impact of starch-tuber formulated to diet behaviour in both sexes, male and female activities varied. Rusdy and Fatmal[16] (2008) suggested that diet activity in males was lower than females due mainly to foraging and copulation activities while females preferred quiet stay for nesting but female activity was very rare to foraging.

3.4 Testing mortality (%) and durability

Giving different dosage of yam-tuber to white mice caused varied mortality. No specimens were killed in the trial G_0 (control). Overall, in the trial G_1 (300 g yam-tuber), all specimens were just apparently killed in day-12 but in day-6, up to 20% of specimens were killed. This finding suggested that specimens in the trial G1 seemed likely to have durability till the end of observation. insignificant mortality rate of specimens throughout 12 days was slightly caused by inappropriate dosage to kill rapidly specimens. This indicated that 300 g yam-tuber was seemingly to have a lower lethal dosage. In contrast to trial G1, Trials of G2 and G3 showed significant mortality rate even in short period. By looking at the trial G_2 (600 g Starch-tuber), more rapid death of mice was seen than trial G1 and control. About 6.67% of specimens damaged in day 4 before 100% death was seen in day 9. In contrast, the trial G_3 (900 g) significantly influenced. For instance, just day 5 after applied, 100% specimens were killed. It is assumed that *knock down* effect was seen in this trial (G3). The more concentration (g) of yam-tuber was applied to specimens, the faster lethal impact (rapid mortality rate) occurred (Table 3).

In the fresh yam-tuber, 469.5 ppm Hydrogen Cyanide (HCN) is found. Meanwhile FAO suggests that safety recommendation of consumption of 50 pm HCN is still allowed[1] (Ditjenbun-Direktorat Perlindungan Perkebunan, 2013). Assessed of wild yam (*Dioscorin* spp.) in Nepal and found about 6.0 ppm cyanogens or HCN content. HCN-free or bonded shape is well known as sianogenic glicoside that cause an intervention of O_2 transportation flow system through the layers[12] Bhandari and Kawabata (2005). Once oxygen flow intervention occurs, a significant breath dysfunction appears followed by paralysis and death[9] (Prichard, 2007). Rapid death effect seen (day 2 after application) with 100% mortality rate occurred (day 5) was in the trial G3. This finding indicated that there was a significant effect of dioscorin properties such as HCN, Alkaloid and lysis enzymes and concentration level given in the baits because in the G3 treatment, much higher concentration (900 g yam-tuber flour) of dioscorin was treated than other trials. Alkaloid in dioscorin causes anticholinesterase. If the dioscorin is available in certain tissue, cholinesterase is not capable of lysis arc chemical transmitter (known as acethylcholine). As a consequence, acethylcholine accumulation in between synapses occurs followed by the increase of transmission stimulation that can cause a simultaneous contraction in the breath muscles. Paralysis, breath dysfunction and death were soon inevitable.

Apart from negative effect of dioscorin, extreme bitter taste in yam-tuber might contribute as well to metabolism instability of mice tasters resulting anti-feeding and food poisoning effects. It was evidenced that the high solution of *D. hispida* tuber extracted with methanol results in 21.5 mg yellowish white solution impacting to food abandonment[17] (Santi S, 2010). A Principle bitter taste in yam-tuber is analysed that the main cause of bitterness is furanoid norditerpenes (diosbulbins A and B)[12] (Bhandari & Kawabata, 2005).

Tabel 1 : The characteristics of white mice performed during entire observation

Dosage	Characteristic behaviours of specimen per day				
	1	2-4	5-6	7-11	12-13
G0	Normal	Normal	Normal	Normal	Normal
G1	• Active movement, • Adaptable to new environment and food, • started to consume cube bait	• Slow movement • Quiet stay • Confusedness • Rather shock • Normal diet behaviour • Less waste	• Rough hair and stand up • Hair fall • Difficult breath	• Slow movement and less appetite in males • Female death	• Entire specimen dead
G2	• Active movement • Adaptable to new environment and food • Started to consume block bait •	• Less waste • Slow movement • Quiet stay • Confusedness • Rather shock • Normal diet behavior	• Rough hair and stand up • Hair fall • Difficult breath • Female death	• Male death	----
G3	• Active movement • Adaptable to new environment and food • Started to consume block bait	• Slow movement • Quiet stay • confusedness • Rather shock • Less appetite • Female death	• Male death	---	----

Tabel 2: The average of bait (cube shape) was eaten by male and female

Sex	Dosages			
	(G_0)	(G_1)	(G_2)	(G_3)
Males	2.51^a	2.35^a	1.94^b	1.81^b
Females	2.94^a	2.31^a	2.00^b	1.87^b

Statistically, number followed by the same word in a row is non-significant based on 0.05 turkey test

Tabel 3 : Effect of varied dosage of yam-tuber to mortality rate was performed

Dosage	Mortality (%) interval per day											
	1	2	3	4	5	6	7	8	9	10	11	12
G_0	0	0	0	0	0	0	0	0	0	0	0	0
G_1	0	0	0	0	0	20	40	60	60	60	60	100
G_2	0	0	0	6.67	13.33	40	66.67	66.67	100			
G_3	0	13.33	20	40	100							

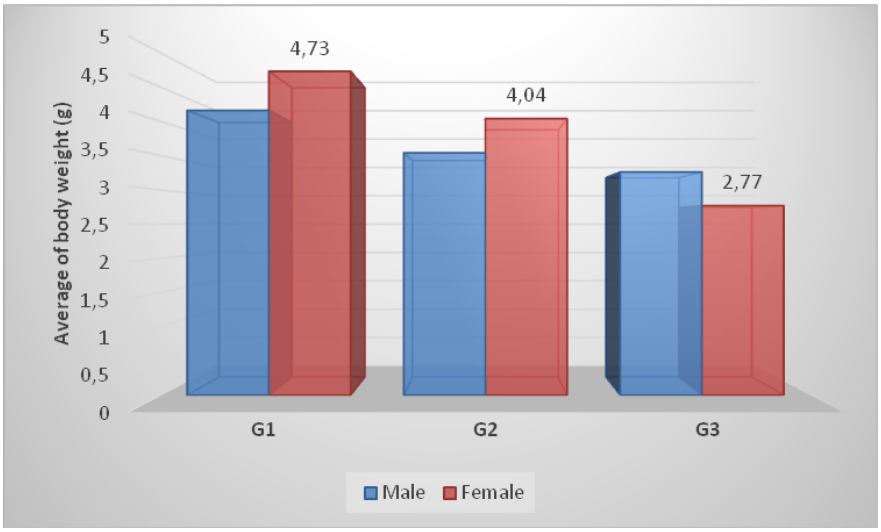

Fig. 1 : The average loss of body weight in the trials in both sexes was performed.

4. CONCLUSION

Generally, mice behaviours investigated from day 2 to day 6 showed slow movement, quiet stay, less appetite, shock, paralysis and death. It was evidenced that mice specimens had negative effect in terms of food consumption, body weight and mortality. The higher dosage given in the trial, the more significant poisonous effect to mice occurred. in male consumption test, the average of bait weight remaining in every dosage given was 2.31 g (300 g), 1.94 g (600 g) and 1.81 g (900 g), while in female, bait remaining was 2.31 g, 2.00 g and 1.87 g respectively. The average of body weight in male in every dosage was 4.16 g (300g), 3.54 g (600 g) and 3.26 g (900 g) and in female was 4.73 g (300 g), 4.04 g (600 g) and 2.77 g (900 g) respectively. the initial death of mice seen was in day 6 (300 g), day 4 (600 g) and day 2 (900 g) but then 100% mortality rate occurred in day 12 (300 g), and day 5 (600 g and 900 g).

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6. CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES

1. Ditjenbun-Direktorat Perlindungan Perkebunan. D. P. (Producer). Kendalikan tikus dengan umbi Gadung; 2013. Retrieved from http://ditjenbun.deptan.go.id/perlindungan/berita-181-kendalikan-tikus-dengan-umbi-gadung.html
2. Hakim, Rahim, Ibrahim, & Beddu. Padi Puso di Kabupaten Pinrang akibat Serangan Tikus; 2016.
3. Priyambodo S. Pengendalian Hama Tikus Terpadu. Penebar Swadaya, Jakarta, 2003

4. Han C, Lin Y, Lee T, Huang W, Lin S, & Hou W. Effects of yam tuber protein, dioscorin, on attenuating oxidative status and learning dysfunction in d-galactose-induced BALB/c mice food and chemical toxicology. An International Journal Published for the British Industrial Biological Research Association 2014; 65: 356-363. doi:doi:10.1016/j.fct.2014.01.012
5. Kumoro AC, & Hartati I. Microwave assisted extraction of dioscorin from gadung (*Dioscorea Hispida* Dennst) Tuber Flour. Procedia Chemistry 2015; 14: 47-55. doi:http://dx.doi.org/10.1016/j.proche.2015.03.009
6. Maspary. Gadung sebagai insektisida nabati; 2012. Retrieved from http://www.gerbangpertanian.com/2012/04/gadung-sebagai-insektisida-nabati.html
7. Azman I, Mutalib SA, et al. Novel *Dioscorea hispida* starch-based hydrogels and their beneficial use as disinfectant. Journal of Bioactive and Compatible Polymers 2016; 31(1): 42-59. doi:DOI: 10.1177/0883911515597704
8. Kardian. Pestisida Nabati Ramuan dan Aplikasi. PT Penebar Swadaya, Jakarta, 2001; 44-49
9. Prichard JD. Hydrogen cyanide toxicological overview: CHAPD HQ, HPA version 2, 2007.
10. Murjani D. Pengujian umbi gadung (*Dioscorea hispida* dennst.) sebagai rodentisida botanis siap pakai dalam pengendalian tikus rumah (*Rattus rattus diardii* linn.) dan tikus sawah (*Rattus argentiventer* rob. & klo.). Master Thesis, Institut Pertanian Bogor, Bogor, 2011.
11. Sukarsa E. Tanaman gadung; 2010. http://www.bbpp-lembang.info/index.php/arsip/artikel/artikel-pertanian/504-tanaman-gadung. Diakses pada tanggal 18 Februari 2014.
12. Bhandari MR, & Kawabata J. Bitterness and toxicity in wild yam (*Dioscorea* spp.) tubers of nepal. Plant Foods for Human Nutrition 2005; 60 (3): 129-135. doi:doi:10.1007/s11130-005-6841-1
13. Nagai TN, Suzuki Y, Tanoue N, Kai, & Nagashima T. (2007). Antioxidant and antihypertensive activities of autolysate and enzymatic hydrolysates from yam (*Dioscorea opposita* Thunb.) ichyoimo tubers. Journal of food, Agriculture & Environment 2007; 5(3&4): 64-68.
14. Nababan R. Uji efektifitas pemberian beberapa pakan tambahan penyedap pada rodentisida nabati ubi gadung (*Dioscorea hispida* dennst.) terhadap tikus sawah (Rattus argentiventer rob. & klo.) *di laboratorium*. Retrieved from Jurusan Ilmu Hama dan Penyakit Tumbuhan Fakultas Pertanian. Universitas Sumatera Utara Medan. (Abstrak), 2012.
15. Suriani N. Gangguan metabolisme karbohidrat pada diabetes melitus. Master Thesis, Brawijaya University, Malang, 2012.
16. Rusdy A, & Fatmal I. Preferensi tikus (*Rattus argentiventer*) terhadap jenis umpan pada tanaman padi sawah. Jurnal Floratek Jurusan HPT Fakultas Pertanian Unsyiah Kuala Banda Aceh 2008; 3: 68-73.
17. Santi SR. Senyawa aktif antimakan dari umbi gadung (*Dioscorea hispida* dennst.). Jurusan kimia FMIPA Universitas Udayana Bukit Jimbaran. Jurnal kimia 2010; 4(1): 71-78.