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THE BIOLOGICAL ACTIVITY OF KUMCHURA RHIZOME TO MELON FLY : II. METHANOLIC KUMCHURA EXTRACT BIOACTIVITY

AKTIVITAS BIOLOGIS RIMPANG KENCUR TERHADAP LALAT BUAH MELON : II. BIOAKTIVITAS EKSTRAK METANOL RIMPANG

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INTISARI

Rimpang kencur diketahui memiliki bioaktivitas terhadap lalat buah kukurbit dalam bentuk gerusan langsung. Bahan ekstrak rimpang juga diuji terhadap telur dan larva lalat buah kukurbit Bactrocera cucurbitae *Coq. Uji toksisitas dilakukan dengan mengencerkan ekstrak metanol rimpang dan mencampurkannya dengan pakan larva pada seri konsentrasi tertentu. Berdasar uji toksisitas ditentukan konsentrasi subletal yang berpengaruh terhadap kelulushidupan larva dan telur, lama stadium larva, berat dan panjang pupa, repelensi ekstrak terhadap larva dan aktivitas hormonal ekstrak terhadap larva. Hasil pengamatan menunjukkan bahwa ekstrak kencur bersifat "sedikit beracun", namun konsentrasi di bawah 0,3125% (untuk telur) dan 2,5% (untuk larva) secara nyata mempengaruhi kelulushidupan lalat, sementara konsentrasi yang sama untuk telur dan 0,625% untuk larva secara nyata memperpanjang lama stadium larva. Repelensi ekstrak terhadap larva terbukti nyata pada konsentrasi sampai 0,3125%, namun tidak terlihat pengaruh ekstrak terhadap berat dan panjang pupa, serta tidak dijumpai adanya aktivitas hormonal ekstrak terhadap lalat.*

Kata kunci : ekstrak kencur, lalat buah melon, bioaktivitas

ABSTRACT

Kumchura (*Kaempferia galanga* L.) rhizome has been known to posses bioactivity to melon fly in its crude form. Extract preparation from the same plant part was tested against melon fly *Bactrocera cucurbitae* Coquillet's eggs and larvae to investigate its toxicity and activity. Toxicity test was done by diluting the rhizome's methanolic extract and incorporating the solution to larval diet. Based on the toxicity test, sublethal concentrations were then tested to determine the extract activity to egg and larval survivals, larval stage duration, puparial weight and length, extracts' repellency to larvae and extracts' hormonal activity to larvae. The result showed that kumchura extract toxicity was only considered "slightly toxic", but sublethal concentrations as low as 0.3125% (to eggs) and 2.5% (to larvae) significantly affected the fly's survival, while the same concentration to egg and 0.625% concentration to larvae significantly prolonged larval stage durations. Extracts' repellency to larvae was significant in sublethal concentration as low as 0.3125%, but kumchura extract has no significant effect on puparial weight and length, and did not contain any hormonal activities toward melon fly.

Key words: kumchura extract, melon fly, bioactivity

INTRODUCTION

Kumchura (*Kaempferia galanga* L.), a rhizome producing plant of the family Zingiberaceae, is a common medicinal plants known throughout Asia and the Pacific. The ability of kumchura rhizome to be anti-insect agent was known since the Ming dynasty in China (1368 - 1644) (Smith and Stuart, 1973). Its use, however, so far is only limited in crude

form. The study of kumchura *in extractio* is still in development. As its activity toward organisms, Kiuchi *et al.* (1988) proved that kumchura rhizomes' methanolic extract was larvicidal to the dog roundworm, *Toxophtera canis* L. Kosuge *et al.* (1985) found similar activity toward He La cells. However, not much was known about the effect of kumchura extract on insect.

In this study, which complement crude kumchura biological activity test previously described (Martono, 1997), the biological activity of methanolic kumchura extract was determined on the melon fly, *Bactrocera cucurbitae* Coquillet. LD50 values for the melon fly were established in order to obtain sublethal doses suitable for testing against melon fly eggs and larvae.

MATERIALS AND METHODS

Insects. Melon flies, larval standard diet (Tanaka *et al.*, 1969) and adult diet were obtained from the USDA Tropical Fruit and Vegetable Research Laboratory in Manoa, Honolulu. Adult flies were fed protein-sugar hydrolysate mixture (3:1, w/w) and water.

Kumchura. Kumchura rhizomes were purchased from local market in Yogyakarta, Indonesia. Rhizomes were grown in the same locale. They were washed, sliced and sun-dried until the water content was at minimum (weight reduced to 25 - 40%). Kumchura extract was obtained by methanolic extraction of sliced and sun-dried rhizomes using the method developed by Kosuge *et al.* (1985).

Toxicity against melon fly eggs and larvae. *Toxicity tests against eggs and larvae.* Dilutions of 100%, 50%, 25% and 12.5% extract in acetone were made, and 5 ml of the solution was mixed with larval diet. The mixed diets were used as hatching media for the eggs and rearing media for the larvae. Twenty eggs and twenty first-instar larvae were used for each treatment, with four replicates per treatment. A check treatment using acetone only as diet-mix was also implemented. The mortality data established a concentration-curve from which a sublethal concentration could be determined, and used as the basis for further experiment(s).

Sublethal concentration effect on melon fly eggs and larvae. *Eggs and larval feeding tests.* Four different sublethal concentrations were prepared. Five ml of each solution were mixed with 100 g larval diet. Melon fly eggs and first instar larvae (20 each, four replicates for each mixture) were placed on the diet-mixes. Development from larva to adult were followed to note any effects on the flies. The criteria used were: egg survival (percentage of egg hatching), larval survival (percentage of larvae pupariating), average number of days to reach puparial stage, puparial weight and length, and puparial survival (percentage of adult emerging).

Larval repellency test. Four different sublethal concentrations were prepared. Five ml of each solution were mixed with 100 g larval diet. About 5 to 10 g of treated larval diet were placed side by side with untreated (acetone mixed) diet in a Petri dish. Care was taken so that the two diets did not mix. Twenty 1-hour-old eggs were then placed on wet cloth or filter paper between those diets. The number of larvae which migrated to the treated or untreated diet after the eggs hatched were recorded. Four replicates were used for each treatment.

Extract hormonal activity. A sublethal dose of the kumchura extract were applied topically to immature stages of the melon fly. The development of these stages was observed, and a development scale (Mandhava, 1986) was used to evaluate the degree of morphological changes.

Data analysis. Data recorded were analyzed using SAS computer program (SAS, 1986) to obtain the LD50 and significancy of the

treatments; while graphs and charts were generated in Sigmaplot.

RESULT AND DISCUSSION

Toxicity against melon fly eggs and larvae. *Toxicity tests to eggs and larvae.* Extraction from dried rhizomes yields brownish liquid with strong kumchura odor. The extract concentration was expressed as the percentage of kumchura extract in acetone (v/v). Five ml of the acetone solution was added to 100 g of larval diet, *i.e.* the actual amount of the kumchura extract per g diet equaled the weight of extract in solution divided by 100. The extract density was 0.98 g/ml, therefore a dose of 5 ml 100% solution in 100 g diet equaled to 0.049 g/g diet or 49 mg/g diet.

The result of the toxicity tests to both eggs and larvae are expressed as LC50/LD50 and LC25/LD25 values and presented in Table 1. The toxicity of kumchura extract to the eggs and larvae were high although still considered "slightly toxic", with LD50 values of 1.5 and 3 mg/g diet, respectively. Study of the sublethal effects of kumchura extract were based on these LD50s.

Table 1. Toxicity of the kumchura extract against eggs and larvae of melon fly

Stages	LC50	LD50	LC25	LD25
Egg	3.101	1.519	1.948	0.955
Larvae	6.202	3.039	3.896	1.909

Values are the result of probit analysis. LC50 unit is percent extract in acetone. LD50 unit is 5 ml extract in acetone added to 100 g diet.

Sublethal effect on eggs and larvae. *Eggs and larvae feeding test.* The percentage of egg hatch, pupation, and adult emergence of melon flies reared from eggs exposed to diet treated with kumchura extract were reduced and similar phenomenon was also observed on melon flies originated from first instar larvae reared in kumchura extract treated media (Table 2). The ability of kumchura extract to reduce the number of egg hatch seemed to correspond with its LC and LD values, as the media for egg hatching was found to be toxic to the egg, especially for the 1.25% and 2.5% concentrations, whose effects were significant compared to the control. Between the concentrations itself, no significant difference was found, meaning that any concentrations as low as 0.3125% would be able to reduce the number of egg hatching.

On the effect of extract to the number of pupation, both treatments to eggs and to larvae show a similar trend. Number of larvae pupating from eggs under treatment was reduced as low as 15.513% (with 2.5% extract concentration); while those from larvae reared on treated diet the number of larvae pupariating reach 43.558% (with 5% extract concentration). This fact shows that although the trend is similar, extract treatment starting from eggs has better effect in reducing larval pupation, as smaller concentration yielded higher pupation failure. Early exposure of kumchura extract to melon flies proved to be more effective, which would be advantageous for its use in protecting products.

The same can also be said to the extracts effect to adults emergence. Both melon flies reared since eggs and since larvae show low percentages of adult emergence. These percentages, especially in the higher concentrations (1.25% to 5%) significantly differ from adult emergence of untreated/control batches. Once again, treating melon flies starting from eggs yield better result in inhibiting adult emergence. Only 12.613% adult emergence was observed when melon flies' eggs were treated with 2.5% kumchura extract.

Table 2. Percentages of hatch, pupation and adult emergence of melon flies reared on concentrations of kumchura extract both from eggs and larvae

Concentration (%)	% hatch egg	% pupation		% adult emergence	
		egg	larvae	egg	larvae
0	79.80 a	73.23 a	85.25 a	70.76 a	72.99 a
0.3125	60.70 a	38.20 ab	-	33.17 b	-
0.625	54.60 ab	37.65 ab	63.61 a	33.33 b	45.85 a
1.25	44.25 b	33.82 ab	53.14 a	24.68 b	46.47 a
2.5	27.50 b	15.51 b	47.39 b	12.61 b	35.54 b
5	-	-	43.56 b	-	33.74 b

Egg treatments started at .3125% conc., larvae treatments at .625% conc. Values represents the means of four replicates after transformation to arcsine x. Values in a column with the same letters are not significantly different (DMRT, for eggs Fhs=8.268; Fpu=10.159; Fem=14.371; df=4.15; $p<0.01$, for larvae, Fpu=32.29; Fem=47.5; df=4.18; $p<0.01$).

Regarding the influence of the kumchura extract to the duration of the larval stadium and the dimensions of the pupae (weights and lengths), Table 3 and 4 list the figures which show that the concentrations had little, if any, effects on the dimensions, and did not differ significantly with the control treatment. But the duration of the larval stadium lengthened significantly. This effect clearly proved that kumchura extract contains compound(s) which inhibit growth, as was observed by Kiuchi *et al.* (1988).

It seemed that although the weight and length of pupae both from egg-reared and larvae-reared batches underwent decreasing effect of the extract, the individual changes were too erratic as seen from the high standard deviation values. The values of weight and length of any pupae treated and reared from any of the two origins therefore did not show significant differences.

Table 3. Duration of larval development, average pupal weight and length of melon flies reared from eggs

Concentration (%)	Duration (days)	Weight (g)	Length (mm)
0.0	7.435± 0.30a	0.013± 0.002a	5.175± 0.27a
0.3125	8.125± 0.25 b	0.010± 0.005a	5.000± 0.91a
0.625	8.136± 0.10 b	0.010± 0.004a	5.125± 0.48a
1.25	8.106± 0.14 b	0.010± 0.004a	4.800± 0.25a
2.5	8.100± 0.08 b	0.010± 0.003a	4.925± 0.10a

Values represents the means of four replications and their standard deviations. Values in the same column with the same letter are not significantly different (DMRT, F=9.715,df=4,15,$p<0.01$ for duration; ANOVA, Fw=0.385,Fl=0.542, df=4,15,$p<0.01$ for dimensions).

Table 4. Duration of larval development, average pupal weight and length of melon flies reared from first instar larvae

Concentration (%)	Duration (days)	Weight (g)	Length (mm)
0.0	6.905± 0.09a	0.014± 0.004a	5.450± 0.53a
0.625	8.406± 0.19 b	0.013± 0.003a	5.200± 0.25a
1.25	8.673± 0.11 b	0.012± 0.005a	5.100± 0.30a
2.5	8.540± 0.22 b	0.012± 0.006a	5.000± 1.05a
5.0	8.745± 0.10 b	0.011± 0.004a	4.850± 0.60a

Values represents the means of four replications and their standard deviations. Values in the same column with the same letter are not significantly different (DMRT, F=2.657,df=4,15,p<0.05 for duration; ANOVA, Fw=0.813, Fl=1.224, df=4,15,p<0.01 for dimensions)

Larval repellency test. The test shows that the higher the concentrations, the more repellent the extract become (Figure 1) Although there were no significant differences between any untreated branches of the olfactometer, in the higher concentrations (2.5 and 5%) there were significant differences between treated and untreated branches of the olfactometer. Larvae would choose to stay in untreated diets when there are kumchura extract in them, regardless of the amount. This test, however, need more elaboration since some of the larvae used were either missing or killed.

Extract hormonal activity. The extract did not inflict any hormonal action toward larvae and pupae, as no morphological change was observed on the test insects.

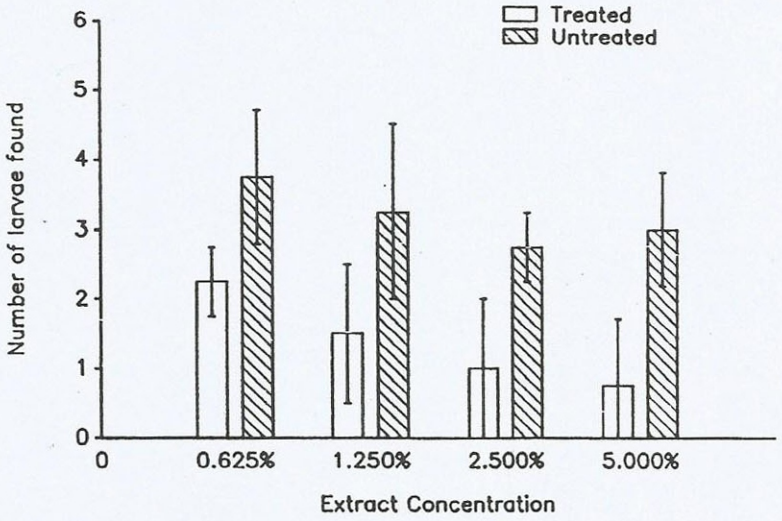

Figure 1. Repellency of kumchura extract to melon fly larvae. Average of four replications.

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REFERENCES

Kiuchi, F., N. Nakamura, Y. Tsuda, K. Kondo & H. Yoshimura. 1988. Studies on Crude Drugs Effective on Visceral Larva Migrants. II. Larvicidal Principles in *Kaempferia galanga* L. *Chem. Pharm. Bull.* 36: 412-5.

Kosuge, T,, M. Yokota, K. Sugiyama, T. Yamamoto, M.Y. Ni & S.C. Yan. 1985. Cytotoxic activities of the Rhizomes of *Kaempferia galanga* L. *Chem. Pharm Bull.* 33:5565-7

Mandhava, B. 1986. *CRC Handbook of Natural Pesticides, Vol. II: Isolation and Identification.* CRC, Boca Raton, Florida, 460p.

Martono, E. 1997. The Biological Activity of Kumchura Extract to Melon Fly. I. Toxicity of Crude Kumchura to Eggs and Larvae. *J. Ind. Plant Protection*, 2(2):27-32

Smith, F.P. & G.A. Stuart. 1973. *Chinese Medical Herbs,* translated from Li Shih Chen's Peng ts'ao kang mu. Georgetown Press: San Francisco. 467 p.

Tanaka, N. I.F. Steiner, K. Ohinata & R. Okamoto. 1969. Low cost larval rearing medium for mass production of oriental and mediterranean fruit flies. *J. Econ. Entomol.* 62:967-8.