Application of Neem Extracts to Prevent Housefly Worms on Salted Fish(การ,ประยุกต์สารสกัดจากสะเดาเพื่อป้องกันหนอนแมลงวันในการผลิตปลา...

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ABSTRACT: Neem seed extracts were studied on the reduction of housefly worms on dried salted fish. Water extract of neem seed (5, 8, 10, 20 or 25 percent) and brine extract of neem seed (1, 2, 3, 6, 10, 15 or 20 percent) were applied to fish fillets and the fillets were introduced to cages containing 15 pairs of houseflies for 1 day. The number of worms and pupa was counted at 48 hours and 72 hours, respectively. The results showed that the extracts affected progeny development of housefly but none of them was good enough to produce worm-free salted fish. However, the number of larva on brine extract experiment were less than those of the water extract one. Salting in 3%, 6%, 10%, 15% and 20% of brine extract, most of the larva failed to molt to the next instar stage. The addition of sesame oil to brine extract of neem seed had no effect on the progeny development of housefly. In field evaluation, the number of insect arrival and the number of larva were inversely proportional to the concentrations of water extract applied on the fish fillets. Control fish became heavily infested with larvae during drying process while larval infestation was less on the treated fish. It is suggested that the water extract should be at least 10% of neem seed in order to decrease larval infestation. However, it was not possible to determine the degree of protection provided by water extract in this study because it depended on many other factors such as weather, light and humidity.

KEY WORDS: neem *(Azadirachta indica),* dried salted fish, housefly *(Musca domestica),* natural insecticide

INTRODUCTION

Dried salted fish are produced and consumed widely in Southeast Asia due to the simple process, low investment and acceptable flavour (1). The main problem encountered the production is insect (such as housefly) infestation during processing and storage. Many salted fish processors responded to the problem by applying dipterax (pyrimiphos-methyl) and left insecticide residues (2). It is, therefore, necessary to find an alternative effective methods of infestation reduction of dried salted fish from natural products. The use of natural products readily available in the tropics and subtropics will reduce the need for insecticides and thereby gradually increase self sufficiency in developing countries. Many researches reported on the extracts of neem tree *(Azadiracta indica)* as insect growth inhibitor and feeding deterrent. Thus, in this report an application of neem seed extracts on the reduction of housefly worms on traditionally salted dried fish was studied.

MATERIALS AND METHODS

Natural pesticide: Neem seeds *(Azadirachta indica* var siamensis) were obtained from the Royal Forest Department, Ministry of Agriculture and Co-operative. The decorticated seeds with fungi were discarded; then, they were kept in bags with good ventilation. The seeds were weighed and ground to be fine powder and were extracted with different solutions described below.

Cages: Each cage was constructed of aluminum frame measuring 31 x 31 x 31 cm^3 supporting a fine nylon mesh. There was an entrance in the front part with 14 cm in diameter at the center carrying 53 cm sleeve attached with a secure knot. Cages were used for maintaining the stock of flies for routine experiments.

Animal: Housefly was used throughout the study. The fly is the most widespread aboundant synanthropic species as well as the most important public health problem. The initial stock culture of housefly was obtained from the Division of Medical Entomology, Department of Medical Science, Ministry of Public Health. The culture established from *Musca domestica* adult or pupa and were maintained under laboratory condition at room temperature as suggested by the supplier. Adult flies were raised on milk-sugar diet (milk:sugar ratio 3:1 moisturized with water) in separated cups. Diet was changed every two days. Cup of fish powder medium (25 g plus 30 ml water in each cup) was used as an oviposition medium. Eggs were laid approximately 54 h after fertilization and hatched to give rise to larva at 8 h later. About 600-800 newly hatched larva were transfered to stainless steel tray containing 18 g of Nestum^{TM}, 12 g of yeast, 20 g of rice bran and 1000 ml of water and then it was covered with chaff about 2.5" in depth. The nursing material was covered with thin cloth to protect undesirable insect infestation. The duration of larva development lasted about 4-5 days. The late third larva creeped up to pupate in layer of the chaff. The larva were collected from the chaff by sieving method or floating method when almost all of them became pupa. Then they were transfered to nylon cages. The newly adults emerged 2–3 days later and were ready for the experiments.

Salted fish: Pla-chon or Snake-headed fish *(Channa striatus)* was selected as the oviposition/feeding medium. The fish was bought fresh and sacrificed imme-diately. It was gutted, beheaded and cut (approximate weight was 18–20 g each) into fillets large enough to be vulnerable for fly infestation during processing. The fillets were salted in 10% brine solution for 16 h as suggested by Joe and Carrie (3) and Hodges (4) in order to produce lightly salted fish and had little effect on the total amount of larval growth.

Preparation of Test Solutions

Water extract solutions: Dried neem seed powder was weighed and stirred with 300 ml distilled water in order to obtained various concentrations of the extract as shown in Figure 1 for 3 h and left at room temperature for 24 h; then, on the following day it was stirred for an addition 1 h. The obtained slurry was filtered through linen cloth. Each extract was a coarse and yellowish green colour solution with strong aroma.

Brine extract solutions: Dried neem seed was weighed and stirred with 10% brine solution in order to obtain different concentrations indicated in Figure 1. Preparation of the solutions was the same as of water extract solutions.

Ovipositional deterrence and inhibition of adult emergence: Ovipositional deterrence and inhibition of adult emergence were performed according to Figure 2. Since housefly is social insect, it was found that once a female fly starts ovipositing on a particular piece of food the others quickly join and commence oviposition. The oviposition resulted in a large mass of eggs being deposited on a single food, whereas the other remained free of eggs (2). In order to prevent the unexpected result such as only one fillet was attacked by most of the flies, an individual fish fillet was introduced to the flies in each cage. Fifteen pairs of five-day-old houseflies were randomly chosen from the stock culture. They were transfered to a cage containing one fish fillet treated with specified test solutions as an oviposited medium. Sucrose and water were fed *ad libitum*. Effects of test solutions were performed as indicated in Figure 2.

Interactive effect of sesame oil on brine extract solutions: Experiments to investigate the synergistic effect of sesame seed oil on brine-testing solutions were performed according to Figure 3.

Field evaluation: Pla-too or Scombar fish *Rastrelliger brachysoma)* was submerged in 10% brine solution for 16 h and partly dried at room temperature. It was, then, dipped into testing solution as indicated in Figure 4. The treated fish were sun-dried for 8 h/day.

Assessment on the effect of neem seed solution as a repellent of flies from salted fish was monitored by taking instantaneous counts of flies touched the fish. The number of alighted insects on the control and treated fish were recorded every 15 min.

Infestation assessment method: Larva infestation was assessed according to the following grading: 0 (zero) indicated that no larva present, L (light) indicated that occasional larva present, M (moderate) indicated that numerous larva but no feeding packs present and H (heavy) indicated that numerous larva and feeding packs present.

Statistical analysis: The data obtained in the laboratory were subjected to analysis of variance. Differences between the means were compared using Duncan's Multiple Range Test (DMRT). The standard deviation of the mean (SD) was used as a measure of the reproducibility and dispersion of results. Interaction of factors (between sesame seed oil and brine extract of neem seed) was investigated by two way analysis of variance. The data obtained from field study were subject to the Kruskal-Wallis test and proportion test. Differences between treatment were compared using Duncan's Multiple Range test.

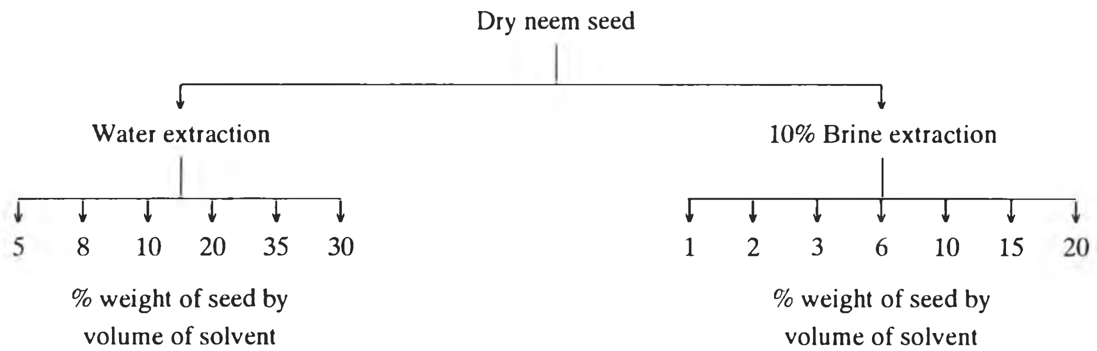

Figure 1 Establishment of different test solutions

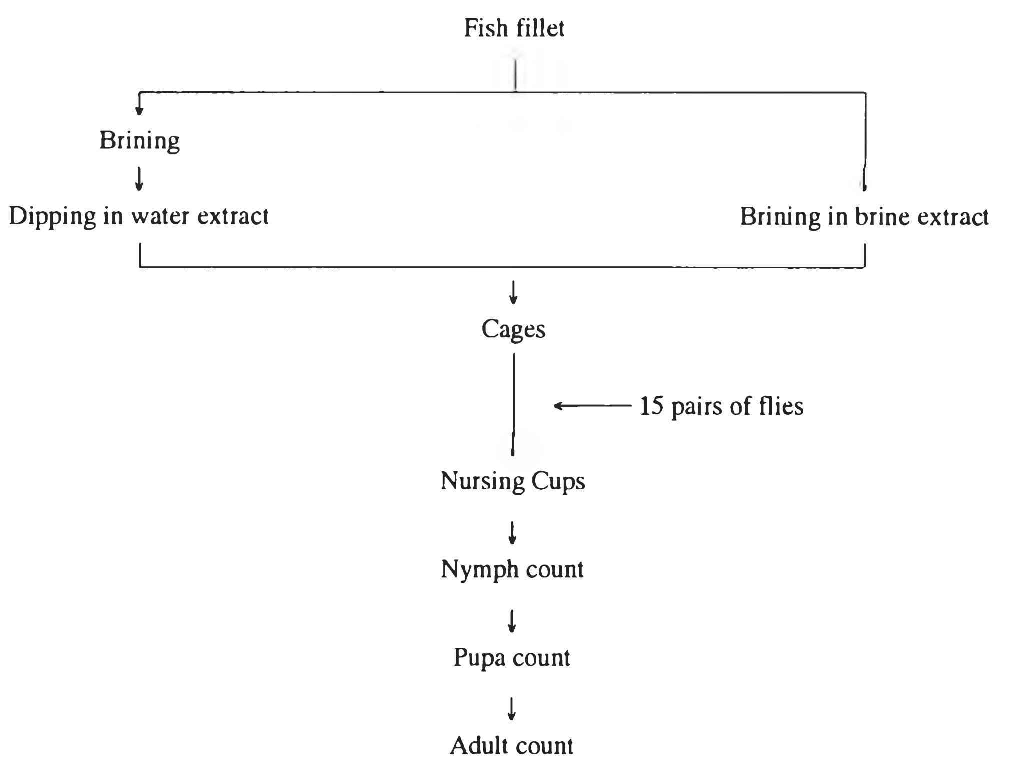

Figure 2 Steps in ovipositional deterrence and inhibition of adult emergence test

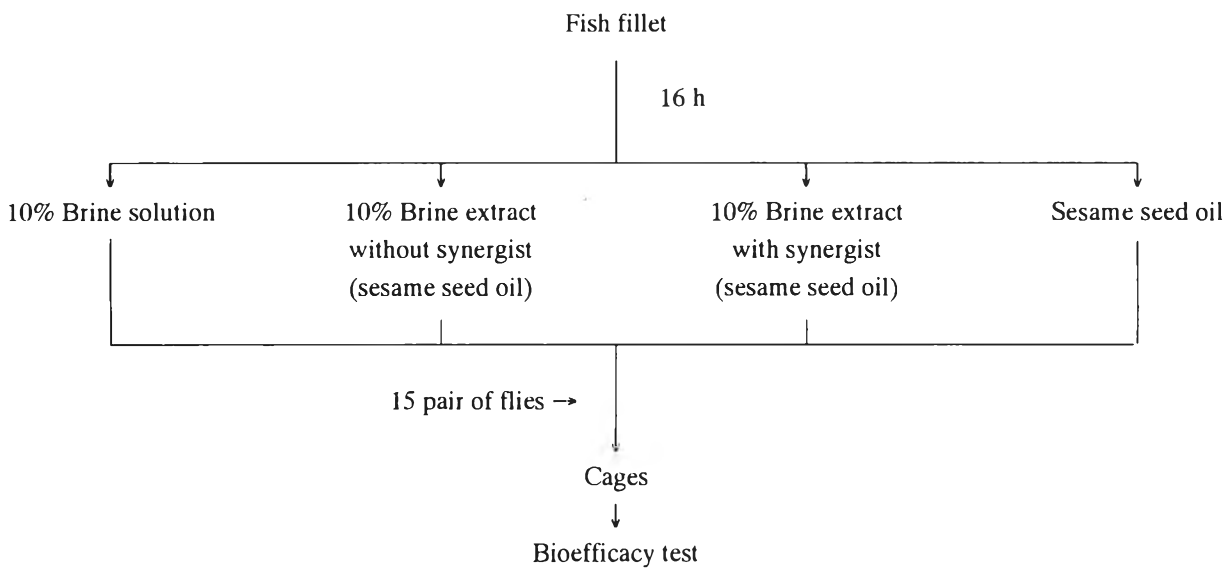

Figure 3 Interactive effects of sesame oil on brine-testing solution

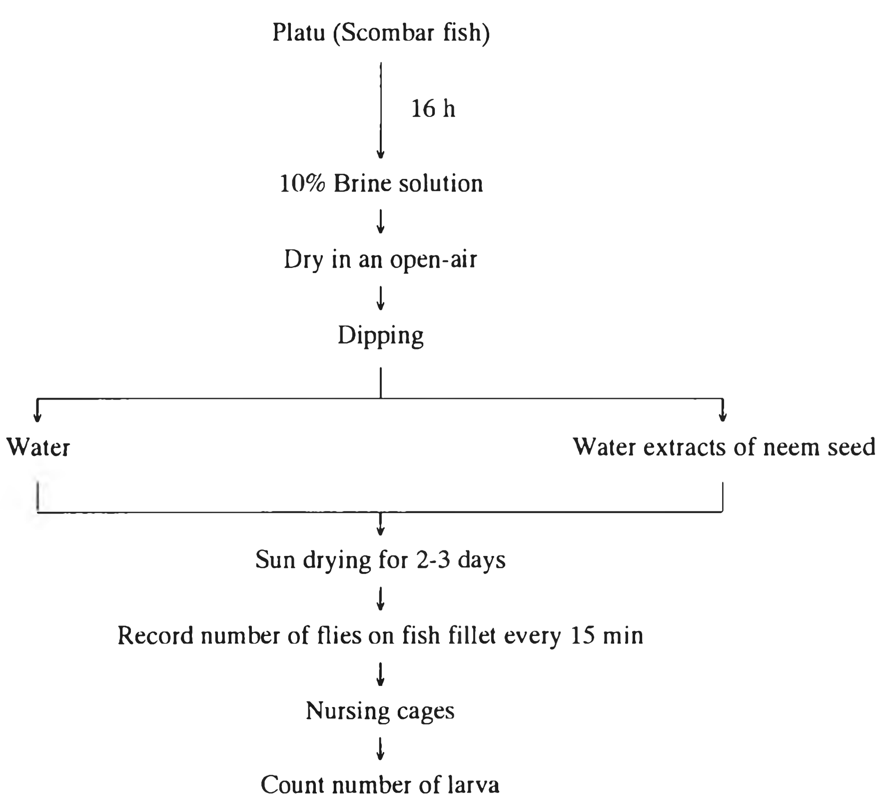

Figure 4 Field evaluation of neem seed extracts on salted fish

RESULTS

Ovipositional deterrence and inhibition of adult emergence

Effect of the water extract: Fish fillets dipped in various concentrations of water extract of neem seed were subject to the oviposited medium of flies. Twenty four hours later, the examination on the number of emergent larva was performed. They were raised on the same substrate until they hatched to be adult larva and flies. The number of emergent larva (L_1), prepupae (L_3) and flies were inversely proportional to the concentrations of the water extract applied to the salted fish fillets (Table 1). Calculation using the figures in this table shows that the concentrations of 5%, 8%, 10%, 20%, 25% and 30% of neem seed water extract reduced the emergent larva by 46%, 49%, 62%, 81%, 80% and 87% compared to that of the untreated control, respectively. With respected to the concentrations of the extract stated above, only 75%, 81%, 80%, 72%, 68% and 50% of the emergent larva developed to be prepupae compared to 79% of the untreated group and 50%, 55%, 34%, 23%, 19% and 0% of the prepupae developed to be adult flies compared to 69% of the untreated control. Thus, the water extract of neem seed showed some degree of protection from housefly worms infestation on salted fish; however, no worm-free salted fish was obtained.

Effect of the brine extract: The number of emergent larva was recorded on the brine extract treated fish fillets. It was inversely proportional to the concentrations of the brine extract (Table 2). By calculation, the concentrations of 6%, 10%, 15%, and 20% of neem seed in brine extract respectively reduced the emergent larva by 58%, 84%, 84% and 98% compared to that of the untreated control. The number of adult larva was decreased dramatically when the concentration of test compound reached 6%. At this concentration housefly could oviposited; however, the progenies hatched out were tiny in shape and died within a short period of time.

Interactive effects between sesame seed oil and brine extract: In order to control the infestation of housefly by using neem seed extract at a lower concentration than that of the above experiment, sesame seed oil was added. Tables 3 and 4 show that the brine extracts had no effect on the number of emergent larva but they affected on the number of prepupae. Sesame seed oil itself affected the number of emergent and prepupae. It expressed with dose response relationship. Analysis of variance demonstrated no interaction between the two compounds.

Field evaluation on insect infestation: The effectiveness of the water extract of neem seed on the reduction of fly worms on sun-dried salted fish was described. The arrivals of fly on the treated salted fish were fewer than those of the control fish (Table 5). The insect arrival decreased progressively with respect to the concentrations of the extract. However, variation on the number of larva in each treatment was recorded as shown in Table 6. The number of larva in the treated group was slightly decreased in some experiment compared to the untreated control; however, no significant difference was detected. The larval infestation during drying process was a consequence of the fly activity and the variation of the weather. It was shown that control fish became heavily infested with larva during drying process; on the other hand, the infestation was reduced on the treated fish (Table 7).

DISCUSSION

Effect of neem seed extracts on the progeny development of housefly: No concentration of neem seed extracts either water extract or brine extract was effective to produce worm-free salted fish. However, the emergent larva of brine extract treated fish had poor development with high mortality in the period between prepupae and pupae. Sombatsiri (5) found that neem extracts had no effect on eggs but survived larva was the target of the neem activity. He found that small number of larva failed to molt to the next instar stage eg. L1–L2 or L2–L3. Schmutter (6) suggested that the neem kernel extracts may act by interfering with the molt-regulating endocrine system. Wilp (7) pointed out that the addition of azadirachtin (the active compound of neem seed) to the food of blowfly larva *(Phormiaterrae novae)* and housefly larva *(Musca domestica)* showed an antifeedant effect and resulted in a slow growth rate. A higher intake of azadirachtin was correlated with a further decrease in weight gain and an increase the mortality rate during larval period. Bidmon *et al.* (8) found that azadirachtin caused a delay in pupariation of larva when they were injected in the first half of the last larval instar of the blue blowfly *(Colliphora vacina)* but no effect was obtained when they were injected at the late instar. The reduction of pupal weight and inhibition of adult emergence was also detected. Adults emerging from azadirachtin-treated larva were smaller with malformations.

Warthen and Uebel (9) suggested that the mortality of the insect was due to starvation rather toxicity since toxic effects seemed to be immediate. Rembold *et al.* (10) suggested that the highest mortality was immediately before larval-pupal ecdysis, indicating a distance in metamorphosis. Slama (11) suggested that growth disrupting activity attributed to neem seed extracts or azadirachtin could, in some cases, be due to their antifeeding action, as antifeedants could also cause developmental deviations that may suggest disturbance of the neuroendocrine system. It was observed that no parent flies died. Sombatsiri (5) suggested that neem seed extracts had little effect against parent flies.

Interactive effects between sesame seed oil and brine extract: It was shown that the brine extract had no effect on the number of emergent larva; however, the number of prepupae was reduced. The results confirmed the work of Sombatsiri (5) who suggested that the neem extract expressed its action after the eggs developed into larva. Sesame oil itself affected on the number of emergent larva and prepupae. The results expressed with dose response relationship. Many workers suggested that sesame oil was effective against insects. Doharey *et al.* (12) found that female *Callosobruchus chinensis* and *Callosobruchus maculatus* did not oviposit on seeds of greengram *(Vigna radiata)* treated with 1% sesame oil and at 0.1%, 0.5% and 1% sesame oil prevented emergence of both species. Singal and Singh (13) found that sesame oil used as surface protectants at 1, 3 and 5 ml/kg of seed to test their efficacy against *Callosobruchus chinensis* on a variety of chickpea *(Cicer arietinum)* resulted in significantly less oviposition on treated than on untrated seeds. It adversely affected hatching and development of embryos, resulting in further population suppression. Choudhary (14) found that sesame oils at 0.5 and 1.0 ml/100 g of chickpea seed *(Cicer arietinum)* reduced damage by *Callosobruchus chinensis.* Skukla *et al.* (15) found that sesame oil reduced oviposition by *Callosobruchus maculatus.* Sesame oil was very effective in controlling weevil populations (16). Shukla *et al.* (15) found that the sesame oil caused 90.48% mortality on *Sitophilu oryzae* and 56.67% mortality on *Rhyzopertha dominica.* However, it was unaffected on *Tribolium castaneum.*

The analysis of variance of the results of this experiment demonstrated no interaction between the two compounds. Bowers (17) found that the naturally occuring synergists sesamin and sesamolin isolated from sesame oil were nearly inactive (sesamin had slightly activity at 100 g) against *Tenebrio molitor* (L.) pupae and the last instar nymphs of the milkweed bug *(Oncopeltus fasciatus Dallas).* Karr *et al.* (18) found that the effect of d-linomene, an insecticidal monoterpenoid, on lethality in earthworm *(Eisenia fetida Savigny)* were not significantly synergized by either piperonyl butoxide or sesame oil.

The present results were contradict to the work of Lee *et al.* (19) who found that one of the minor constituents of sesame oil, sesamin, was used to synergize the effect of pyrethrin, in addition of a small quantity of this substance markedly increased the effectiveness of fly sprays. Islam (20) found that the addition of sesame oil to methyl-tertiary butyl-ether (MTB) and methanolic extracts of *Azadirachta indica, Melia azadarach, Amoora rohituka, Annona reticulata* and *Annona squamosa* resulted in higher mortality of *Callosobrucus chinensis* and the *Dicladispa armigera* and increased deterrent effects.

Field evaluation on the protection of insect infestation by the water extract: The present results of field study showed that the number of insect arrival and the number of larva were inversely proportional to the concentrations of water extract applied on the salted dried fish. In addition, control dried salted fish became heavily infested with larva during drying process. On the other hand, larval infestation was lower on neem seed treated fish. The result showed that no concentration of water extract was effective to obtain worm-free dried salted fish.

It was indicated that the water extract did not afford sufficient protection on housefly worm infestation to the sample. It is suggested that the water extract of neem did not penetrate to the fish tissue. By the nature, female flies often crawl into small crevics and, by the use of their ovipositor, deposit the eggs still further into the mass. And when the embryological development is complete, logitudinal spilt develops in the egg shell and the young larva emerges (21).

It was not possible to determine the degree of protection provided by the water extract in the field because it depended on multifactor. Esser and Hanson (2) suggested that fly activity seemed to be influenced by weather changes and the concentrations of the water extract applied on salted fish. On the hot and sunny day, it was found that the fish were dried rapidly and fly activities were low; thus, the concentrations of the water extract needed were lower than those on the dull and humid days. In the latter situation, the fish would be liable to fly infestation. The freshness of fish was also important in salted dried fish processing. It was suggested that insect infestation on the fish was reduced if the raw material was fresh.

CONCLUSION AND SUGGESTION

Significant reduction of housefly worms on the fish salted in the brine extract of need seed are shown in the present experiment. However, it is required high concentrations of the extract to obtain the result as such. None of the concentrations of the extracts water extract and brine extract, was effective to produce worm-free salted fish. Larva on the treated fish failed to mold to the next instar stage eg. L1–L2 or L2–L3.

To enhance the effectiveness of the salting, sesame seed oil was added to the brine extract. Synergistic effect of sesame oil on the progeny development of housefly was not obtained. Interestingly, sesame seed oil itself affected the progeny development of housefly.

An application of water extract on dried salted fish in the conventional method demonstrated that the number of insect arrival and the number of larva were inversely proportional to the concentrations of the extract. Unfortunately, it was impossible to determine the degree of protection provided by the extract because environmental factors such as temperature and humidity must be concerned.

Further studies to improve the extraction, using suitable solvents or combining with other plant extracts are recommended. It is also necessary to persuade the use of other natural synergists as well as perform a taste panel on the organoleptic of the treated products. The dipping time of the salted fish into the extract or the salting time if the extract is prepared as a brine solution is also required to be investigated in order to reduce the neem concentration in the extract.

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Table 1 Effects of the water extract of neem seed on progeny development of housefly. Data expressed as mean ± SD and the number of sample is in parenthesis. Figures indicated by the same letter in the same column are not significantly different at $p < 0.05$ as determined by DMRT.

Conc. of extr. (w/v)	**Emergent larva** (L1)	**prepupae** (L3)	**Adult**
0%	395 ± 91^{a} (42)	310 ± 76^{a} (42)	215 ± 50^{a} (33)
5%	215 ± 38^{b} (3)	160 ± 38^{b} (3)	$80 \pm 15^{b,d}$ (3)
8%	202 ± 26^{b} (3)	163 ± 17^{b} (3)	90 ± 18^{b} (3)
10%	$151 \pm 35^{b,c}$ (3)	$120 \pm 23^{b,c}$ (3)	$41 \pm 7^{b,c,d}$ (3)
20%	$74 \pm 36^{b,c}$ (3)	$53 \pm 24^{b,c}$ (3)	$12 \pm 4^{c,d}$ (3)
25%	$80 \pm 21^{b,c}$ (3)	$54 \pm 17^{b,c}$ (3)	$10 \pm 7^{c,d}$ (3)
30%	52 ± 13^{c} (3)	26 ± 14^{c} (3)	0^{c} (3)

Table 2 Effect of Brine extract of neem seed on progeny development of housefly. Data expressed as mean ± SD and the number of sample is in parenthesis. Figures indicated by the same letter in the same column are not significantly different at $p < 0.05$ as determined by DMRT.

Conc. of extr. (w/v)	Emergent larva (L1)	prepupae (L3)	Adult
0%	395 ± 91[a]	310 ± 76[a]	215 ± 50[a]
	(42)	(42)	(33)
1%	435 ± 56[a]	296 ± 27[a]	136 ± 19[b]
	(6)	(6)	(6)
2%	398 ± 32	271 ± 22	28 ± 12
	(9)	(9)	(9)
3%	327 ± 75	1 ± 5	0
	(12)	(12)	(12)
6%	167 ± 64	0	0
	(15)	(15)	(15)
10%	144 ± 35[c, d]	0[b]	0[c]
	(3)	(3)	(3)
15%	63 ± 17[d, e]	1 ± 2[b]	0[c]
	(3)	(3)	(3)
20%	4 ± 7[c]	0[b]	0[c]
	(3)	(3)	(3)

Table 3 Sesame seed oil effect on brine extract of neem seed on the number of the emergent housely larva. Data expressed as mean ± SD and number of sample is in parenthesis.

	conc. of sesame seed oil (w/v)\		
conc. of extr. (w/v)	0%	0.5%	1%
0%	395 ± 91	241 ± 26	93 ± 57
	(42)	(3)	(3)
1%	435 ± 56	255 ± 15	26 ± 16
	(6)	(3)	(3)
2%	375 ± 32	203 ± 61	16 ± 16
	(9)	(3)	(3)

Table 4 Sesame seed oil effect on action of brine extract of neem seed on the number of prepupae of housefly. Data expressed as mean ± SD and number of sample in parenthesis.

	conc. of sesame seed oil (w/v)		
conc. of extr. (w/v)	**0%**	**0.5%**	**1%**
0%	310 ± 75	172 ± 14	40 ± 30
	(42)	(3)	(3)
1%	296 ± 27	94 ± 10	3 ± 0
	(6)	(3)	(3)
2%	261 ± 22	30 ± 21	0
	(9)	(3)	(3)

Table 5 Orientational response* of fly allowed a choice between treated and control dried salted fish. Figures in the same column indicated by the same letter are not significantly different at $p < 0.05$ as determined by K-W test.

	Arrival on water extract treated dried salted fish (mean rank)		
Concentration (w/v)	**1st experiment**	**2nd experiment**	**3rd experiment**
0%	122.97[a]	100.75[a]	78.46[a]
10%	94.53[b]	60.86[b]	52.86[b]
30%	46..61[c]	36.22[c]	43.66[b]
40%	32.21[d]	44..17[c]	27.02[c]

* The number of insects alighting on the control and treated dried salted fish was recorded from 7 fillets.

Table 6 Effect of the water extract of neem seed on the number of larva on dried salted fish. Figures indicated by the same letter in the same column are not significantly different at $p < 0.05$ as determined by K-W test.

	Mean rank of the number of larvae*						
conc. of extract	**1st experiment**	**2nd experiment**	**3rd experiment**	**4th experiment**	**5th experiment**	**6th experiment**	**7th experiment**
0%	22.36[a]	19.71[a]	21.29[a]	21.93[a]	11.86[a]	10.86[a]	12.43[a]
5%	ND	ND	ND	ND	12.43[a]	18.00[b]	8.74[a]
10%	15.43[b]	16.36[a]	15.00[b]	ND	8.71[a]	4.14[c]	11.79[a]
30%	9.86[c]	13.21[a]	13.57[b]	12.50[b]	ND	ND	ND
40%	10.36[c]	8.71[a]	8.14[c]	11.93[b]	ND	ND	ND
50%	ND	ND	ND	11.64[b]	ND	ND	ND

* mean rank from 7 pieces; ND = not determine

Table 7 Effect of the water extract of neem seed on larval infestation. Data expressed as number of fish fillet infested by larva of fly (O = none, L = < 10 larva, M = 10-50 larva, H = > 50 larva, ND = not determined). Figures indicated by the same letter in the same column are not significantly different at $p < 0.05$ as determined by proportion test.

Treatment	**Number of fish fillet infested**																				
	1st experiment			**2nd experiment**			**3rd experiment**			**4th experiment**			**5th experiment**			**6th experiment**			**7th experiment**		
(% w/v)	**O**	**L**	**M-H**	**O**	**L**	**M-H**	**O**	**L**	**M-H**	**O**	**L**	**M-H**	**O**	**L**	**M-H**	**O**	**L**	**M-H**	**O**	**L**	**M-H**
0%	1^{a}	2^{a}	3^{a}	0^{a}	4^{a}	3^{a}	0^{a}	3^{a}	4^{a}	0^{a}	2^{ab}	5^{a}	0^{a}	2^{ab}	5^{a}	0^{a}	5^{a}	2^{a}	3^{a}	2^{a}	2^{a}
5%	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1^{b}	1^{a}	5^{a}	0^{a}	0^{b}	7^{b}	5^{a}	2^{a}	0^{b}
10%	3^{b}	3^{a}	1^{b}	2^{b}	3^{ab}	2^{a}	2^{b}	3^{a}	2^{ab}	ND	ND	ND	1^{b}	4^{b}	2^{b}	1^{b}	6^{a}	0^{c}	3^{a}	4^{a}	0^{b}
30%	6^{C}	1^{a}	0^{c}	3^{bc}	2^{ab}	2^{a}	3^{b}	2^{a}	2^{ab}	2	4	1	ND	ND	ND	ND	ND	ND	ND	ND	ND
40%	6^{c}	1^{a}	0^{c}	5^{c}	1^{b}	1^{a}	6^{c}	0^{b}	1^{b}	4^{b}	2^{ab}	1^{b}	ND	ND	ND	ND	ND	ND	ND	ND	ND
50%	ND	ND	ND	ND	ND	ND	ND	ND	ND	4^{b}	1^{b}	2^{b}	ND	ND	ND	ND	ND	ND	ND	ND	ND

REFERENCES

1. K. Suphonhong. Effects of modified atmosphere packaging on storage stability of dried salted fish Sardine *(Sardinops neopil Chardus). Proceedings of the Seminar on Advances in Fishery Post-harvest Technology in Southeast Asia,* Singapore, 1991 p. 151.
2. J.R. Esser, S.W. Hanson. Prevention of insect infestation and losses of salted dried fish in Indonesia by treatment with an insecticide approval for use on fish. *Proceedings of a symposium held in conjunction with the seventh session of the Indo-Pacific Fishery Commision Working Party on fish technology and marketing,* FAO fisheries report No. 401 supplement, Thailand, 1990 pp. 168-180.
3. M.R. Joe, E.R. Carrie. *Introduction to fish technology,* van Nostrend Reinhold, New York, 1991 pp. 133-135.
4. R.J. Hodges. Biological methods for integrated control of insects and mites in tropical stored products. *Trop. Stroed Prod. Inform.* 48: 9-15 (1984).
5. K. Sombatsiri. Development of neem extracts in the controlling of insect pests under field conditions. *Proceedings of Workshop on Participatory on Farm Experimentation with Botanical,* Sukhothai, Thailand, 1988 pp. 35-43.
6. H. Schmuter. Ten years of neem research in the Federal Republic of Germany. In K.R.S. Schmutterer, A.H. Rembold (eds.), *Natural Pesticides from the Neem Tree (Azadirachta indica A. Juss)* ~ (Proceedings of the first international neem conference. Rottach-Egem, Germany, 1980) German Agency for Technical Cooperation (GTZ), Eschbom, 1982 pp. 21-31.
7. H. Wilp. Growth and adult molting of larvae and pupae of the blowfly Phormiaterrae-novae in relationship to azadirachtin concentrations. In H. Schmutterer, K.R.S. Ascher (eds.). *Natural Pesticides from the Neem Tree (Azadirachta indica A. Juss) and other tropical plants* (Proceedings of the Third International Neem Conference, Nairobi, Kenya, 1986), Dt. Ges. fur Techn. Zusammenarbeit (GTZ) GmbH. Comp, Eschbom, Germeny, 1987 p. 299.
8. H.J. Bidmon, G. Kauser, P. Bobus, J. Koolmaun. Effect on azadirachtin on blowfly larvae and pupae. In H. Schmutterer, K.R.S. Ascher (eds.) *Natural Pesticides from the Neem Tree (Azadirachta indica* A. Juss) *and other tropical plants* (Proceedings of the Third International Neem Conference, Nairobi, Kenya, 1986), Dt. Ges. fur Techn. Zusammenarbeit (GTZ) GmbH. Comp, Eschborn, Germany, 1987 pp. 253-271.
9. J.D. Warthen Jr. E.C. Uebel. Effect of azadirachtin on House Crickets, Acheta domesticus. In K.R.S. Schmutterer, A.H. Rembold (eds.), *Natural Pesticides from the Neem Tree (Azadirachta indica* A. Juss) (Proceedings of the first international neem conference. Rottach-Egem, Germany) German Agency for Technical Cooperation (GTZ), Eschborn, 1982 pp. 137-149.
10. H. Rembold, G.K. Sharma, C.H. Czoppelt. Growth-regulating activity of Azadirachtin in holometabolous insects. In K.R.S. Schmutterer, A.H. Rembold (eds.) *Natural Pesticides from the Neem Tree (Azadirachta indica* A. Juss) (Proceedings of the first international neem conference. Rottach-Egem, Germany) German Agency for Technical Cooperation (GTZ), Eschborn, 1982 pp. 121-129.
11. K. Slama. The principles of antihormone action in insects. *Acta Entomologica* 75: 65-82 (1978).
12. R.B. Doharey, R.N. Katiyar, K.M. Singh. Effect of edible oils in protection of greengram *(Vigna radiata)* seed from pulse beetles *(Callosobrucus chinensis* and *Callosobrucus maculatus). Ind. J. Agric. Sci.* 58(2): 151-154 (1988).
13. S.K. Singal, Z. Singh. Studies of plant oils as surface protectants against pulse beetle. *Trop. Pest. Manage.* 36(3): 314-316 (1990).
14. B.S. Choudhary. Residual effect of eight vegetable oils in chickpea against pulse beetle, *Callosobrucus chinensis* Linn. *Ind. J. Plant Protect.* 18(1): 89-92 (1990).
15. R.M. Shukla, G. Chand, M.L. Saini. Laboratory evaluation of effectiveness of edible oils against three species of stroed grain insects. *Plant Protect. Bull. Faridabad.* 44 (1-2): 14-15 (1992).
16. P. Ran, R.S. Verma, S.V. Singh. Vegetable oils as grain protectant against *Sitophilus oryzae* Linn. *Farm Sci. J.* 3(1): 14-20 (1988).
17. W.S. Bowers. Juvenile hormone activity of natural and synthetic synergists. *Science* 161: 895-897 (1968).
18. L.L. Karr, C.D. Drewes, J.R. Coats. Toxic effects of d-limonene in the earthworm *Eisenia fetida* (Savigny). *Pestic. Biochem. Physiol.* 36(2): 175-186 (1990).
19. B.H. Lee, J.I. Lee, R.K. Park. Current status and perspectives of quality improvement in sesame. *Korean J. Crop Sci.* 33 (supp. 1): 86-97 (1988).
20. B.N. Islam. Use of some extracts from *Meliaceae* and *Annonaceae* for control of rice hispa, *Dicladispa armigera,* and the pulse beetle, *Callosobrucus chinensis.* In H. Schmutterer, K.R.S. Ascher (eds.) *Natural Pesticides from the Neem Tree (Azadirachta indica A. Juss)*

and other tropical plants (Proceedings of the third International Neem Conference, Nairobi, Kenya, 1986), Dt. Ges. fur Techn. Zusammenarbeit (GTZ) GmbH, Eschbom, Germany, 1987, pp. 217-242.

21. J.R. Busvine. *Insects and Hygiene: the Biology and Control of Insect Pests of medical and Domestic Importance,* 3rd. edition., Chapman and Hall, London, 1980 p. 192.

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การประยุกต์สารสกัดจากสะเดาเพื่อป้องกันหนอนแมลงวันในการผลิตปลาเค็ม

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ฝ่ายพิษวิทยาทางอาหารและโภชนาการ สถาบันวิจัยโภชนาการ มหาวิทยาลัยมหิดล ศาลายา นครปฐม 73170

บทคัดย่อ : สารสกัดจากสะเดาถูกนำมาศึกษาเพื่อลดหนอนแมลงวันในการผลิตปลาเค็ม การศึกษาทำโดยชุบปลาเค็มหลังหมักเกลือลงในสารสกัดเมล็ดสะเดาที่ความเข้มข้นร้อยละ (โดยน้ำหนัก) 5, 8, 10, 20, 25 และ 30 หรือหมักปลาในน้ำเกลือที่มีสารสกัดเมล็ดสะเดาที่ความเข้มข้นร้อยละ (โดยน้ำหนัก) 1, 2, 3, 6, 10, 15 และ 20 ในขั้นตอนการทำเค็ม โดยให้แมลงวันบ้านเพศเมียวัยเจริญพันธุ์ตอมปลาเค็มที่ความเข้มข้นดังกล่าวเป็นเวลา 1 คืน แล้วตรวจสอบการเป็นหนอนที่ระยะเวลา 48 ชั่วโมง และ 72 ชั่วโมง จนกระทั่งเป็นตัวเต็มวัย พบว่าสารสกัดเมล็ดสะเดามีผลต่อการเปลี่ยนแปลงในแต่ละระยะของวงจรชีวิตแมลงวันบ้าน โดยเป็นสัดส่วนผกผันกับความเข้มข้นที่นำมาประยุกต์ใช้ อย่างไรก็ตามไม่มีความเข้มข้นของสารสกัดเมล็ดสะเดาใด ๆ ที่สามารถนำมาใช้อย่างมีประสิทธิภาพพอที่ไม่พบหนอนแมลงวันในปลาเค็ม ปลาเค็มที่ถูกหมักในน้ำเกลือที่มีสารสกัดเมล็ดสะเดาจะมีประสิทธิภาพในการเกิดหนอนน้อยกว่าปลาเค็มที่ชุบในสารสกัดเมล็ดสะเดาด้วยน้ำหลังหมักเกลือแล้ว โดยพบว่าปลาเค็มที่ถูกหมักในน้ำเกลือที่มีสารสกัดเมล็ดสะเดาความเข้มข้นร้อยละ (โดยน้ำหนัก) 3, 6, 10, 15, 20 จะมีหนอนระยะแรกเกิดขึ้น แต่หลังจากนั้นไม่สามารถเจริญเติบโตเป็นหนอนระยะต่อไปและตายในที่สุด ในการเพิ่มประสิทธิภาพของสารสกัดเมล็ดสะเดาโดยใช้น้ำมันงานั้นพบว่าไม่มีผล ในการทำปลาเค็มตากแดดพบว่า จำนวนแมลงวันที่มาตอมและจำนวนหนอนเป็นปฏิภาคผกผันกับความเข้มข้นของสารสกัดสะเดาที่ใช้ โดยที่ชิ้นปลาในกลุ่มควบคุมมีการตอมและปริมาณหนอนมาก อย่างไรก็ตามไม่สามารถกำหนดแน่นอนถึงความเข้มข้นที่เหมาะสมได้ในการลดหนอนแมลงวันในปลาเค็มเพราะขึ้นอยู่กับปัจจัยหลายอย่าง เช่น อุณหภูมิ, แสงแดด และความชื้น เป็นต้น

กุญแจคำ : สะเดา *(Azadirachta indica)*, ปลาเค็มตากแห้ง,
แมลงวันบ้าน *(Musca domestica)*, สารกำจัดแมลงธรรมชาติ