INSECTICIDAL ACTIVITIES OF *BARRINGTONIA SARCOSTACHYS* BARK EXTRACT AGAINST CABBAGE HEAD CATERPILLAR *CROCIDOLOMIA PAVONANA* (F.)

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ABSTRACT

Bioassays of *Barringtonia sarcostachys* (Lecythidaceae) bark ethanol extract were conducted in the laboratory to evaluate the effect on mortality, feeding, oviposition, and reproduction against *Crocidolomia pavonana* (Lepidoptera: Pyralidae). Bioassay tests was performed from March – October 2007 using leaf-residual feeding method. The bark extract of *B. sarcostachys* possessed a strong lethal effect against *C. pavonana* larvae with LC_{50} of 0.14% which is equivalent to the lethal effect of neem seed extract with LC_{50} of 0.18%. Extracts (0.8%-0.24%) inhibited feeding *C. pavonana* larvae by as much as 68.79%-99.1%. In that concentration range, the extract also reduced fecundity by 29.6%-69.2%. Lower concentrations (0.14% -1%) inhibited oviposition of the adult female by as much as 65.7%-95.6%. Overall, these activities showed the effectiveness of *B. sarcostachys* bark extract as a botanical insecticide to control *C. pavonana*.

Key words: Botanical insecticides, feeding, lethal effect, oviposition, reproduction

INTRODUCTION

The use of synthetic insecticides has both some advantages and disadvantages, today they are also evidently could generate some disadvantages. Pest resistance, pest resurgence, secondary pest outbreak, environmental pollution, as well as residual hazard that affecting the consumers are examples resulted from improper use of insecticides (Ritter *et al*., 2013). These negative impacts of synthetic insecticides create reverse reaction from the public which ultimately led to doubts in using the synthetic insecticides. The doubt drives persons to seek an alternative ways or an alternative means of pest control. The alternate pest control later is expected to be developed to overcome or at least could reduce the problems of the using of synthetic insecticides and could be applied easily. Related to these requirements, utilization of plant as insecticide is one alternative that could be pursued (Isman, 2006).

Available information suggests that the using of botanical insecticides has several advantages. In the environment, botanical insecticides could be decomposed easily. They are effective against target pests, and are generally fairly safe to the natural enemies of pests and other non-target organism . These advantages are in line with the concept of integrated pest management (IPM), which requires the using of insecticides that are not or smallest rising negative impact on the non-target organisms and the environment (Copping and Menn, 2000; Isman, 2008). A number of plants from various plant families are known to have bioactivity against insects (Ntonifor, 2011). Syahputra *et al*. (2004) reported that some plant species from the Clusiaceae, Lecythidaceae, and Sapindaceae were active against insects. Some crude extracts from Annonaceae, Meliaceae and Piperaceae from Indonesia were reported possess insecticidal activity against Lepidopteran larvae (Leatemia and Isman, 2004a; Prijono *et al*., 2006, Dadang *et al*., 2011).

Indonesian forests are tropical rain forests estimated to store many species of plants which have insecticidal activity. Up to now, the exploration and the empowerment of local plants as sources of botanical insecticides has been being investigated. Exploring the potential of assets are necessary to evaluate the bioactivity of species as potential insecticides plants against agricultural pests. This study was conducted to evaluate the lethal effect and antifeedant activity of stem bark extract of *Barringtonia sarcostachys* (Lecythidaceae) against *Crocidolomia pavonana* (Lepidoptera: Pyralidae) larvae and the inhibition of reproduction and anti-oviposition activity of adult female of *C. pavonana*. *C. pavonana* as a major pest of Brassicaceae crops.

MATERIALS AND METHODS

Test Plant Materials

The bark of *Barringtonia sarcostachys* (Lecythidaceae) was collected in February 2007 from natural forests in Sintang District, West Borneo, Indonesia. The bark was air-dried under ambient conditions in shaded areas (protected from direct exposure of the sunlight).

Test Insects

The test insects; larvae and adult female of *Crocidolomia pavonana* (Lepidoptera: Pyralidae), were reared in the Laboratory of Pesticides, Tanjungpura University, Indonesia. The insect colony was maintained under ambient conditions (25-33 °C, 65-85% RH, and ca. 12 L : 12 D regime). The larvae were fed with pesticide-free broccoli leaves and the adults were fed 10% honey solution in cotton swab.

Extraction

The air dried bark was ground with blender and sieved with 0.5 mm or 1 mm mesh sieves. The ground plant materials were extracted with 98% ethanol by an infusion method with stirring for 24 hours. The extracts were filtered using Whatman no 41 filter paper and the solvent was evaporated by using Eyela N1001S rotary evaporator at a temperature of 55-60 °C. The crude extract obtain was used in bioassay.

Larval Feeding - toxicity

The bioassay was conducted in 2007 at the Laboratorium of Pesticides, Faculty of Agriculture, Tanjungpura University, Indonesia. In this bioassay was used ethanol extract of neem seeds *Azadirachta indica* (Meliaceae) as a positive control. One of the components from neem seeds is azadirachtin that commonly known as plant active components of botanical insecticide commercially (Schmutterer, 1995). Preparation of neem seeds extracts in a similar way to the preparation of *B. sarcostachys* bark extract. Neem seeds were obtained from the neem plant that grows in Pontianak, West Kalimantan, Indonesia. The extracts ware tested against second instar larvae of *C. pavonana* by leaf feeding–method. The extract was diluted with ethanol to the desired concentrations and tested at six concentration levels within ranges of concentrations which were expected to cause between > 0 and < 100% mortality as determined in preliminary tests. The extract preparations were applied uniformly on each side of broccoli leaf discs (three cm diameter) using a microsyringe (25µl side^{-1}) and then air-dried. Control leaf disks were treated with solvent only. Second instar larvae *C. pavonana* were allowed to feed in treated leaves for two days and then were fed untreated leaves until reaching the fourth-instar stage. Seventy to ninety larvae in groups of 15 were used in the test with each extract. The number of dead larvae from the second to fourth instar was recorded daily and the data were analyzed by the probit method via PROC PROBIT of the SAS Package (SAS Institute, 2008).

Larval Feeding - deterrency

The extract was assayed for feeding deterrency using the fourth instar larvae of *C. pavonana* using leaf-disc choice and no-choice tests (Koul, 2005). Extract application and treatment procedures were similar as in bioassay.

Choice test. Extract was tested at concentration of 0.08%, 0.14% and 0.24% (w/v) which were within the range of effective concentration with regard to survival of the test insect from final larval instar to the adult stage. Two treated and two control leaf discs were arranged alternating around the edge of the dish (nine cm diam.) lined with moist towel paper. Five fourth instar larvae (three hr old) were released into each dish. A total of 25 larvae (five larvae/dish) were used in each treatment and fed for 24 hours. The area of treated leaves eaten was estimated using a mm^2 grid paper.

No choice test. This test was done using the same procedures as in the choice test, but in this test treated and control leaf disc were placed in separated dishes. The duration of feeding assay and prosedures for leaf area measurement were the same as in the choice test. This test was arranged in a completely randomized design with 10 replications. Analysis of variance (Steel and Torrie, 1980) was performed to compare the effect of extract concentration on leaf consumption and means between concentration were separated using Tukey's range test using the SAS program (SAS Institute, 2008).

Oviposition

Choice test. Extract was tested at concentration of 0.08%, 0.14% and 0.24% (w v^{-1}) that equivalent to the LC_{25}, LC_{50} and LC_{75} respectively. Two-leaf stages broccoli seedlings were sprayed on both sides of leaves with extract suspension using one litre plastic hand-sprayer commonly used by home gardeners. The extract preparation was prepared with distilled water containing ethanol and emulsifier polyoxyethylene alkylaryl ether (Besmor, a.i. 207.4 g L^{-1}). The concentration of emulsifier used in the study was 0.1% (v v^{-1}). Control seedlings were sprayed with distilled water containing ethanol and emulsifier as above. After spray deposits on leaves were air-dried, two treated and two control seedlings were placed alternatingly at the bottom of a plastic cage (21 cm diameter, 30 cm high) with a gauze top and a plastic base. Five days old two pairs of male and female adults of *C. pavonana* were released into the cage and provided with 10% honey solution in cotton swabs suspended with a string from the top of the cage. After two days, the seedling were removed from the cage and replaced with the same number of freshly-treated and control seedlings. Each treatment was replicated six times. Oviposition tests were run for four days and during the holding period the age of the experimental adult spanned from five to eight days (post emergence). The total number of eggs deposited by the females over four days on treated seedling at particular treatments compared to controls using paired *t*-test ($\alpha = 0.05$).

No choice test. In this test, treated and control seedlings were placed in separate plastic cages (two seedlings per cage). In this test also used five days old two pairs of male and female adults of *C. pavonana*. The experiment was arranged in a completely randomized design with six replications. The number of eggs laid by the females over 4 days in each oviposition unit was counted. The data were analyzed using analysis of variance (Steel and Torrie, 1980) via PROC Anova of the SAS Program (SAS Institute, 2008) and Tukey's range test was used to separate the means.

Reproduction

A total of 300 second-instar larvae in groups of three were offered treated or control leaves for 48 hours, then they were fed with untreated leaves and the survivors were allowed to develop until

the adult stage. It can be noted from the total of larvae that each treatment was expected to give 14 pairs of male and female adults of *C. pavonana* (14 replications). The extract application and treatment procedures were the same as in effect on feeding. Extract was tested at concentration of 0.08%, 0.14% and 0.24% (w/v) that are equivalent to the LC_{25}, LC_{50} and LC_{75} respectively. Adult lifespan and female fecundity of survivors were recorded. Upon emergence, adults were confined in pairs in inverted plastic container (10 cm diameter, 20 cm height) with gauze top and fed 10% aqueous honey solution. Portions of broccoli leaves stemmed (3 cm x 3 cm ca.) were placed inside each container for oviposition. Insects were observed daily and the number of dead adults and egg deposited were counted. The data on the male and female lifespan and female fecundity were subjected to analysis of variance (Steel and Torrie, 1980) via Proc GLM of the SAS Program (SAS Institute, 2008), and means between treatments were compared using Duncan's new multiple range test.

RESULTS AND DISCUSSION

Effect on Mortality

The mortality pattern of larval due to stem bark extract of *B. sarcostachys* is presented in Figure 1. In two days after treatment (DAT), the treatment could cause larval mortality. The two highest level of concentration in the third day showed larval mortality above 80%. Larval mortality was quite high at the beginning of observations and relatively constant in subsequent observations. This mortality pattern indicates that the active compounds contained in extracts of the bark *B. sarcostachys* have a relatively quick in causing mortality of larval *C. pavonana*.

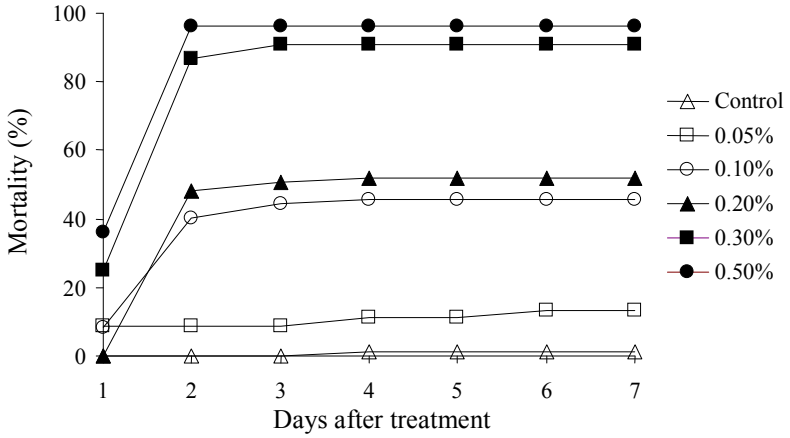

Fig. 1. The pattern of mortality of *C. pavonana* larva treated by *B. sarcostachys* extract

Results of probit analysis showed that the extract of the bark of *B. sarcostachys* possessed LC_{50} values is small, ie 0.14% (Table 1). The LC_{50} values was not significantly different compared to the LC_{50} of *A. indica* seed extract that its value was 0.24%. Slope of the regression line (*b*) of both extracts *B. sarcostachys* and *A. indica* were tested overlap. The small LC_{50} of the extract of *B. sarcostachys* stem bark was an indication that this extract had strong insecticidal activity against *C. pavonana*. Not significantly LC_{50} between the two kinds of extracts indicated that the stem bark extract of *B. sarcostachys* possessed insecticidal activity equivalent to seed extract of *A. indica*. Concerning with the high of insecticidal activity, further study to develope *B. sarcostachys* as sources of botanical insecticides against *C. pavonana* larvae needs to be done.

Table 1. Parameters of concentration-mortality relationships *B. sarcostachys* and *A. indica* extract against larvae of *C. pavonana* [a]

Extracts	*a* ± SE	*b* ± SE	LC_{50} (CI 95%) (%)	LC_{99} (CI 95%) (%)
B. sarcostachys [1]	2.50 ± 0.52	2.94 ± 0.65	0.14 (0.06 – 0.23)	0.87 (0.42 – 37.23)
A. indica [2]	1.91 ± 0.28	3.04 ± 0.34	0.24 (0.19 – 0.35)	1.37 (0.71 – 6.99)

[1] Number of larvae treated and controlled, 346 and 76, repectively
[2] Number of larvae treated and controlled, 372 and 74, repectively
a = intercept of the regression line, *b* = slope of the regression line, SE = Standard Error, CI= Confidential limit

A number of plant extracts have been known to possess a lethal effect against the larvae of *C. pavonana*, including *Piper retrofractum* (Piperaceae), *Aglaia odorata* (Meliaceae), *Annona* spp. (Annonaceae), *Lansium domesticum* (Meliaceae), and *Sandoricum koetjape* (Meliaceae) (Leatemia and Isman, 2004a). Extract of *P. retrofractum* at 0.5% gave 100% mortality against second instars of *C. pavonana* (Prijono *et al*., 2006). While, extracts of *A. odorata* at concentrations of 1% which were assayed in the laboratory gave 92% mortality against same insect target (Dadang *et al*., 2007). A mixture of both extract at a concentration of 0.1% effective in suppressing populations of *C. pavonana* in the field (Dadang *et al*., 2011). Considering the strength of *B. sarcostachys* extract against larval *C. pavonana*, continuing research to develop a plant *B. sarcostachys* as a source of botanical insecticides is feasible to carry out. The phytochemical test showed that the bark extract of *B. sarcostachys* was indicated contain alkaloid, flavonoid, triterpenoid, and saponin. Saponin isolated from seeds of *B. asiatica* active as antifeedant against *Epilachna* sp. larvae (Anthony *et al*., 2002).

Effect on Feeding

Choice test. Treatment of ethanol extract in the range of concentration tested suppressed the larvae feeding activity 68.7% -99.1% (Table 2). The larvae of *C. pavonana* fed the control leaves more than treated leaves. Feeding inhibition could be caused by the presence of biologically active substances in the extract. They could shorten or terminate the larval feeding activity. Inhibitory compounds contained in the extract of *B. sarcostachys* seem to be able to cover or disrupt feeding stimulation signals. Flavonoids from cabbage are feeding stimulants for *Plutella xylostella* larvae (Lepidoptera : Plutellidae) (van Loon *et al*., 2002).

Table 2. Weight of leaves consumed in choice-tests.

Concentration [a] (%) (≈LC)	Weight of the leaves consumed (mg) ± SD [b]	
	Treatment	Control
0.08 (LC_{25})	109.2 ± 104.7 a	589.3 ± 77.53 b
0.14 (LC_{50})	5 ± 9.59 a	651.5 ± 54.73 b
0.24 (LC_{75})	3 ± 4.47 a	646.5 ± 76.8 b

[a] Number of larvae used per concentration of 25. [b] SD: standard deviation.
For each concentration, the average followed by the same letter is not significantly different by paired *t*-test (α = 5%).

No choice test. The tested extracts suppressed 57.6% - 79.2% of the larval feeding activity (Table 3). This suppression is directly correlated with extract concentration. The suppression of feeding activity in this test was lower than in the choice test. In general, it can be said that for both of the choice-test and no choice-test, the ethanol extracts of the bark *B. sarcostachys* demonstrated feeding inhibition. This implies that in the field, the *C. pavonana* larvae will be able to distinguish between treated plants from untreated. This might indicate that the active compound for feeding

inhibition works more as the primary inhibitor. As a result, the tested larvae died by starvation indirectly, however due to low feeding activity, some larvae still survived for a certain time period before they died. When associated with pest control in the field, this situation will give some advantages because the larval that survive can be utilized as prey by predators.

Table 3. Effect of *B. sarcostachys* extract on feeding by no choice-test

Concentration [a] (%)	Average weight of the leaves consumed (mg) ± SD [b]
Control	707.6 ± 91.77 a
0.08 (LC_{25})	300.4 ± 91.27 b
0.14 (LC_{50})	242.2 ± 192.43 b
0.24 (LC_{75})	147.4 ± 116.77 b

[a] Number of larvae used per concentration of 25.

[b] SD: standard deviation.

For each concentration, the average followed by the same letter is not significantly different by Tukey's range test ($\alpha = 5\%$).

In this experiment the inhibitor component in the bark extract of *B. sarcostachys* seems adequate to deter larval feeding. A variety of feeding mechanisms are known for active compounds isolated from other plants (Isman, 2002; Koul, 2005). For the bark of *B. sarcostachys*, their feeding mechanisms should be examined more deeply after the pure component has been identified.

Various extracts or active components from plants that work as feeding inhibitors have been reported (Isman, 2002; Kaur and Sing, 2003; Leatemia and Isman, 2004b; Peng, 2004). Anthraquinone (emodin, citreorosein, and emodic acid) from *Cassia nigricans* (Fabaceae) showed antifeedant activity against *Heliothis virescens* (Lepidoptera: Noctuidae), *Bemisia tabacci* (Homoptera: Aleyurodidai) and *Anopheles gambiaea* larvae (Diptera: Culicidae) (Georges *et al*., 2007). Raja *et al*. (2005) and Pavunraj *et al*. (2006) found that *Hyptis suaveolens* (Lamiaceae) and *Excoecaria agallocha* (Euphorbiaceae) possessed antifeedant activity against Lepidopteran pests. Antifeedant activity of rhein isolated from the ethyl acetate extract of *Cassia fistula* flower (Fabaceae) was observed against *H. armigera* (Noctuidae) (76.13%) at 1000 ppm concentration (Duraipandiyan, 2011). Harun *et al*. (2001) succeeded in isolating eusiderin terpenoids from *Eusideroxylon zwageri* (Lauraceae) (kayu belian: Pontianak, Indonesia), which has strong feeding inhibitor activity against insect *Sparsa epilachna* (Coleoptera: Coccinellidae).

For insect pest management using compounds with feeding inhibition activity, non-toxicity for non-target organisms is exhibited because of their high selectivity. In the field, these compounds can be used for pest control and their applications can be integrated with other control measures in integrated pest management (IPM).

Effect on Oviposition

Choice test. *B. sarcostachys* extract sprayed on broccoli seedlings reduced significantly the number of eggs laid by *C. pavonana* female compared with controls (Table 4) showing a direct correlation with concentration tested. At 0.14%, the number of eggs laid on broccoli seedling was reduced approximately 3 times as compared with controls, while 1% and 2% solutions reduced the number of eggs by eight and 25 times, compared with controls, respectively. The percentage of inhibition of oviposition of the tested extract ranged between 65.7% - 95.6%.

Table 4. Effect of *B. sarcostachys* extract on oviposition by choice-test [a]

Concentration (%) (≈ LC)	Average number of eggs laid ± SD [b]	
	Treatment	Control
0.14 (LC_{50})	143 ± 85.10 a	417.40 ± 112.15 b
1 (LC_{99})	51.17 ± 62.30 a	482.50 ± 170.92 b
2 (2 x LC_{99})	21.17 ± 39.74 a	481.67 ± 97.73 b

[a] The number of females used for each concentration is 18 pairs.
[b] SD : Standard Deviation.
For each concentration, the average followed by the same letter is not significantly different by paired *t*-test (α = 5%).

No choice test. The number of eggs laid on broccoli seedling treated by bark extract of *B. sarcostachys* at 2% (2 x LC_{99}) was significantly different compared to the number of eggs laid in the control (Table 5). In contrast, 0.14% (LC_{50}) and 1% (LC_{99}), did not inhibit oviposition compared with controls. In the range of extract concentration of 0.14% - 2%, egg laying of *C. pavonana* females was inhibited by 1.9% -75.7**%.**

Table 5. Effect of bark extract of *B. sarcostachys* on oviposition by no choice-test [a]

Concentration (%) (≈ LC)	Average number of eggs laid ± SD [b]	Percent inhibition
Control	957.6 ± 150.9 a	-
0.14 (LC_{50})	939.4 ± 228.0 a	1.9
1 (LC_{99})	647.4 ± 304.4 a	32.4
2 (2 x LC_{99})	233.0 ± 121.1 b	75.7

[a] The number of female used for each concentration of 10 pairs.
[b] SD : Standard Deviation.
For each concentration, the average followed by the same letter is not significantly different by Tukey's range test (α = 5%).

The inhibition of *C. pavonana* female oviposition in this experiment showed that the bark extract of *B. sarcostachys* acts as a repellent on broccoli seedlings. Signals coming from the extract can mask the towing signal from broccoli. Allyl isothiocyanate attracts the imago of *C. pavonana* female to lay eggs on broccoli (Honda, 1995). The attraction of female insects to lay eggs is a combination of response to stimuli received by the senses of sight, mechanical, olfactory, and gustatory. Insect olfactory senses detect volatile compounds in the extract, while the mechanical sensory and gustatory detect non-volatile compounds on the leaves. The host selection activities of female insects for laying eggs are guided by chemoreceptors located in the antenna, tarsus, ovipositor or their proboscis (Ryan, 2002; Schoonhoven *et al*., 2005).

Effect on Reproduction

All treatments at all concentrations tested did not influence longevity of *C. pavonana* male (Table 6). In contrast, female longevity was prolonged with 0.08% solution while 0.14% and 0.24% solutions did not prolong female longevity. This situation could happen for the same amount of consumption, because larvae fed treated leaves (0.08%) consumed more of the leaf part which did not contain active components than that which contain active component(s). The accumulation of food consumed during the larval stage is the energy reserve to support the growth and development of adult insects.

The treated substrate that was consumed by larvae could also reduce female fecundity. The 0.08% extract did not decrease fecundity significantly, but at a higher concentration (0.14%), it decreased fecundity significantly by 69%. The decline in fecundity could be due to lower egg production, but not due to its short life span. The highest concentration (0.24%) did not decrease fecundity significantly compared with controls. This condition existed because the larval could survive after consuming the treated leaf making them tolerant, however these were observed to avoid the treated leaf. This situation allowed these selected insects to survive and reproduce normally.

Table 6. Effect of bark extract of *B. sarcostachys* on imago longevity and fecundity [a]

Concentration [b] (%)	Average longevity (days)		Fecundity (eggs/female/day)
	Male	Female	
Control	22.07 a	18.50 b	24.38 a
0.08	18.86 a	21.86 a	16.03 a
0.14	20.29 a	19.43 ab	7.50 b
0.24	18.86 a	19.50 ab	17.16 a

[a] Imago reared from instar II larvae fed with extract.
[b] Each concentration tested used 14 pairs of imago.
For each concentration, the average followed by the same letter is not significantly different by Tukey's range test ($\alpha = 5\%$).

In other studies, the active fraction of *Dysoxylum acutangulum* (Meliaceae) stem bark fed to the second-instar larvae *C. pavonana* at concentrations of 2.54, 3.29 and 3.93 ppm (equivalent to LC_{10}, LC_{30} and LC_{50}) were demonstrated to decrease female fecundity by 44.0, 64.7 and 58.1%, respectively. This effect could be associated with the decrease in protein content of ovaries of the females (29, 47.8 and 42.8% decrease, respectively). Such treatments also delayed the maturity of ovaries of the females (Syahputra *et al*., 2002). The active fraction of *Calophyllum soulattri* (Clusiaceae) stem bark at concentrations of 0.03%, 0.05%, and 0.075% (equivalent to LC_{50}, LC_{85} and LC_{99}) were also demonstrated to decrease the fecundity of adult female by 19.3%-65.6% and delay the maturity of ovaries by 1-3 days (Syahputra, 2007).

CONCLUSION

The bark extract of *B. sarcostachys* possesses strong insecticidal activity against *C. pavonana* larvae that is equivalent to neem seed extract. It also displayed feeding inhibitory activity against *C. pavonana* larvae, reduced oviposition and suppressed reproduction of *C. pavonana* female. For pest management, the synergy of these activities can increase the extract effectiveness. The efficacy of this extracts need to be evaluated in the field.

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