Bioactivity Effect of *Piper nigrum* L. and *Jatropha curcas* L. Extracts Against *Corcyra cephalonica* [Stainton]

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**Abstract**

Petroleum ether extract of black pepper [*Piper nigrum*] and physic nut [*Jatropha curcas*] were shown to have insecticidal efficacies against rice moth [*Corcyra cephalonica*] [Stainton]. The *C. cephalonica* larvae [16 day old] were shown to have similar susceptibilities to petroleum ether extract of *P. nigrum* and *J. curcas* with LC₅₀ values of 12.52 and 13.22 µL/mL, respectively. In a bioassay using no-choice tests, the parameters used to evaluate antifeedant activity were Relative Growth Rate [RGR]; Relative Consumption Rate [RCR], efficiency on conversion of ingested food [ECI] and Feeding Deterrence Indices [FDI]. Both extracts showed high bioactivity at all doses against *C. cephalonica* larvae and antifeedant action was increased with increasing plant extract concentrations. The petroleum ether extract of *P. nigrum* and *J. curcas* showed strong inhibition on egg hatchability and adult emergence of *C. cephalonica* at the lowest concentration. Based on the results of this study petroleum ether extracts of *P. nigrum* and *J. curcas* could be used in IPM program for rice moth.

**Keywords:** Antifeedant; Feeding deterrence; *Jatropha curcas*; *Piper nigrum*; *Corcyra cephalonica*; Egg hatchability; Adult emergence

**Introduction**

The rice moth, *Corcyra cephalonica* [St.] is the major and important pests of stored commodities in the tropics [1], Asia, South America and Africa [2,3]. The larvae feed on rice, corn, cocoa, chocolate, dried fruit, biscuits, coffee and other seeds. The rice moth is a worldwide pest of stored foodstuffs. Control of these insects generally requires the use of chemical insecticides that are toxic to humans and domestic animals and harmful to the environment [4]. In addition the larvae while feeding, leaving silken threads and contaminate the grain by producing dense webbing containing their fecal material and cast skins. The webbing formed is noticeably dense and tough adding the damage caused [5,2]. For the control of stored produced insects, it is frequently more safe to use plant materials with insecticidal, antifeedant or repellent properties than the use synthetic insecticides [6,2] and several plant species have been noted for these purposes in pest management. However, no similar work has been carried out with rice moth, *C. cephalonica*. So black pepper [*Piper nigrum*] and physic nut [*Jatropha curcas*] are some of these plants that may possess insecticidal or antifeedant properties [7,8,9]. In this study we evaluated the efficacy of petroleum ether extract of *P. nigrum* and *J. curcas* against rice moth [*C. cephalonica*] larvae.

**Materials and Methods**

**Insects rearing**

The rice moth [*C. cephalonica*] larvae were obtained from an entomology laboratory stock culture of University Putra Malaysia [UPM] and reared on medium including finely ground rice and maize flour in the ratio 1:1 [w/w] at 27 ± 1°C, 75 ± 5% RH with a 12:12 h light : dark cycle as method of [2] with some modifications. The food media were sterilized in an autoclave before experimentation. The subcultures and the tests were set up under the same conditions. *C. cephalonica* larvae [16 ± 1 days old] was used to the experiments. All the cultures in plastic containers [28×18×18 cm] were held in trays with guards submerged in water to prevent insects from crawling into them [2].

**Plant materials**

Fruits of *P. nigrum* were supplied from Sarawak State in the North-East of Malaysia and seeds of *J. curcas* were prepared from botanical garden in University campus. Plant extracts of *P. nigrum* and *J. curcas* were prepared by the percolation method described by [10]. Fruits of *P. nigrum* and seeds of *J. curcas* were ground to powder using a grinder prior to oil extraction. The powders [300 g] were soaked in methanol [95%] for 48 h, filtered and the residues were extracted afterwards. An equal volume of water added to the crude extract and extraction were done by petroleum ether [11]. The prepared extracts were concentrated by rotary evaporator [40°C] and stored at 4°C for further use.

**Toxicity of plant extracts**

Laboratory bioassays were conducted to evaluate toxicity of petroleum ether extracts of *P. nigrum* and *J. curcas* against *C. cephalonica*. To prepare stock solutions [w/v] of each extract, 10 gram of crude extract was dissolved in 100 ml of respective solvent. Solutions were diluted using the formula $C_1V_1 = C_2V_2$ [12], where $C_1$ and $C_2$ are concentration of 1st and 2nd solution, $V_1$ and $V_2$ are volume of 1st and 2nd solution, respectively. For evaluating efficacy of plant extracts, rice kernels were treated with 2, 4, 6, 8, 10% of prepared dilutions with n-Hexane, then were shaken to ensure uniform coverage of extracts on rice kernels. After shaking, treated rice in conical flask was placed on filter paper to evaporate the solvent. Then the rice kernels were divided five parts by electric balance [each part 5 g]. After that each part was placed in the Petri dishes and 20 larvae [16 ± 1 days old] of *C.
**Evaluation antifeedant**

No-choice test as described by [15] and [16] was carried out to determine antifeedant activity of plant extracts with some modifications. One mL of prepared concentrations of 1, 2, 3, 4, 5% or 2, 4, 6, 8, 10 µL of plant extracts or 1 mL solvent alone as control were applied to 5 gram rice kernels against larvae [16 ± 1 days old] of *C. cephalonica*. After evaporating the solvent, the rice kernels were placed back in Petri dishes [5 cm dia.]. Then ten group-weighted larvae of *C. cephalonica* [starved for 24 hours] were transferred to each pre-weighed rice kernels in Petri dishes. After 3 days of feeding under laboratory conditions [27 ± 1˚C, 75 ± 5% RH with a 12:12 h light: dark cycle], the rice kernels and live insects were re-weighed, and mortality of insects, if any, was recorded. Five replicates of each treatment were prepared including the control. Weight loss and nutritional indices were calculated as described by [17,15].

The following parameters were calculated: Weight Loss [%WL] = [(IW-FW)×100/IW], where the IW is the initial weight and FW is the final weight; Relative Growth Rate [RGR] = (A – B) / (B × day), where A is weight of live larvae on the third day [mg] / no. of live larvae on the third day, B is original weight of live larvae [mg] / original no. of larvae; Relative Consumption Rate [RCR] = D / (B × day), where D is biomass ingested [mg] / no. of live larvae on the third day; Efficiency of Conversion of Ingested food [ECI] [%] = [RGR / RCR] × 100. The antifeedant action was evaluated by calculating the Feeding Deterrence Index [FDI] by formula, FDI [%] = [(C – T) / C] × 100, where C is the consumption of control rice kernels, and T is the consumption of treated rice kernels [18,19]. The mortality data were adjusted for mortality in the control using Abbott’s formula and expressed as percentages [20]. Results of nutritional studies were expressed as means ± SEM and the significance of mean difference between treatments and control was assessed using the analysis of variance procedure at 5% probability level with individual pair wise comparisons with Tukey’s test using the SAS v. 9.1.3 software package in Microsoft Windows 7 [21].

**Effect of plant extracts on egg hatchability**

Five grams treated rice with the petroleum ether extracts of *P. nigrum* and *J. curcas* at the 1, 2, 3, 4 and 5% dose level were placed in plastic Petri dishes [5 cm dia.] and twenty uncapsulated eggs [0-24 h old] of *C. cephalonica* were introduced into each Petri dish using a fine brush. Five replicates of each treatment and untreated rice kernel were set up. After one week, Petri dishes were checked for eggs that failed to hatch [3] and percentage egg hatch was calculated. Results were analyzed by ANOVA and mean values were adjusted by Tukey’s comparison test.

**Evaluation adult emergence**

Rice kernels [5 g] were treated with plant extracts at 1, 2, 3, 4, 5% and solvent only as a control. Then impregnated rice were placed in 25 ml Petri dishes and 20 larvae [3rd instar] of *C. cephalonica* were allowed to feed on to produce adults. Number of adults emerged [2] was recorded at the end of the experiment. Five replicates of each treatment and untreated rice kernel were set up. Data were analyzed by using analysis of variance [ANOVA] and Tukey’s Multiple Comparison Test was used for means and comparison of means.

### Results

**Efficacy of plant materials against *Corcyra cephalonica***

Experiments were carried out to evaluate insecticidal activities of plant extracts from fruits of *P. nigrum* and seeds of *J. curcas* against *C. cephalonica* larvae. The medium lethal concentration [LC50] of plant extracts on the *C. cephalonica* larvae 72 hours from commencement of exposure are presented in Table 1. Results also showed that the petroleum ether extracts from fruits of *P. nigrum* and seeds of *J. curcas* had LC50 values of 12.5 and 13.2 µL/mL against *C. cephalonica* larvae, respectively. Overlapping of the 95% fiducial limits showed that differences between the applied extracts against *C. cephalonica* larvae were non-significant. Petroleum ether extracts of *P. nigrum* and *J. curcas* at 12 µL/mL dose level caused mortality against *C. cephalonica* larvae with values of 65.6 and 66.5%, respectively. While, high mortality was observed at 20 µL/mL dose level with values of 88.9 and 98.0%, respectively (Figure 1).

**Evaluation antifeedant efficacy of plant materials against *C. cephalonica***

The results showed reduction in RGR, RCR and ECI of *C. cephalonica* larvae when the rice kernels treated with *P. nigrum* and *J. curcas* extracts and significantly reduced the RGR of *C. cephalonica* larvae at the lowest concentration dose level of 2 µL/g rice kernels (Table 2). Feeding Deterrence Indices [FDI] showed that the plant extracts had antifeedant action against *C. cephalonica* larvae at all concentrations. Also, petroleum ether extracts of *P. nigrum* and *J. curcas* showed antifeedant action against *C. cephalonica* larvae at a concentration of 6 µL/g rice kernels, with 55.87 and 48.08% reduction in feeding, respectively (Table 2). All the treatments showed significant
weight loss in rice kernels because of feeding by C. cephalonica larvae. Also the weight loss [%] due to C. cephalonica larvae feeding on treated rice kernels with petroleum ether extracts of P. nigrum and J. curcas were significantly different at dose level of 8 µL/g compared with the control (Table 2). Petroleum ether extract of P. nigrum and J. curcas were the most effective treatment. This agreed with [22] who showed that neem leaf powder, nochi leaf powder and neem oil are effective in controlling the rice moth [C. cephalonica] in groundnut kernels and pods. [23] Showed that Acauras calamus essential oils are effective on C. cephalonica larvae. In another study, [24] evaluated the effect of neem oil volatiles by confusing the adults and larvae of C. cephalonica in a chamber containing neem oil. They recorded a marked decline in the reproductive potential and egg hatchability.

Effect of plant materials on egg hatchability

Results in Table 3 showed that egg hatchability was reduced by petroleum ether extract of P. nigrum and J. curcas at the lowest dose level [2 µL/mL] with values of 59 and 58%, respectively [Table 3]. Significant reductions in egg hatchability revealed the harmful effects of petroleum ether extracts of P. nigrum and J. curcas towards C. cephalonica eggs. This observation is in agreement with that of [25] who reported that food treated with Jatropha gossypifolia seed extract strongly inhibit the fecundity of Tribolium castaneum compared with Tribolium confusum at doses of 8000 and 16000 ppm. Bunker and Bhargava determined the effect of vegetable oils on the eggs of C. cephalonica. The treatments were castor bean [Ricinus communis], coconut [Cocos nucifera], groundnut [Arachis hypogaea], Indian mustard [Brassica juncea], sesame [Sesamum indicum], and sunflower [Helianthus annuus] oils at 0.5, 1.0, 2.0, 3.0 and 5.0%. All the vegetable oil concentrations were significantly superior to the control in reducing egg hatchability. In this study the percentage of egg hatch inhibition in all the treatments increased with an increase in concentration.

Effect of plant materials on adult produce

The number of adults of C. cephalonica that emerged from the treated rice kernels decreased with increasing concentration of plant extracts. All the treatments strongly suppressed adult emergence of C. cephalonica larvae at the lowest concentrations with 2 µL/mL dosage (Table 4). The mean percent of F1 adults of C. cephalonica that emerged from the treated rice kernels strongly decreased even at the lowest concentrations of petroleum ether extracts from P. nigrum and J. curcas. These observations are in agreement with [26] who showed that the J. curcas oil at the lowest concentration of 0.5% suppressed adult emergence in Callosobruchus maculatus, also indicating that the oil had ovicidal activity. The adults of C. cephalonica that emerged from rice kernels that treated with petroleum ether extract of P. nigrum and J. curcas at the lowest concentration [2 µL/mL] were 3 and 8% compare with untreated rice kernels with 86 and 85%, respectively. In this regards, [22] reported on the efficacy of a range of plant products, including neem leaf powder and edible oil in protecting stored groundnuts against the rice moth [C. cephalonica] and noted that even though all the plant products and edible oils afforded protection, neem leaf powder and neem oil were most effective. Allotey [2] studied on groundnut kernels treated with Citrus sinensis at dosage of 0.5 g and 2.0 g per 40 g of legume seeds, but differences between botanicals were not significant when all dose levels were considered. They reported that Eichhornia crassipes suppressed the emergence of C. cephalonica to a greater extent at dosage of 0.5, 1.0 and 2.0 g than Citrus sinensis and Chromolaena odorata.

Discussion

The Piperaceae family has been reported to have insecticidal activities due to presence of many potential phytoto-chemicals. The P. nigrum extracts offer a unique and beneficial source of bio-pesticide material for the control of insect pests on a small scale [9,27]. The major components of P. nigrum fruit extracts such as piperine, carophyllene and limonene are reported as having insecticidal properties. Many insecticidal components of plant extracts are mainly monoterpene such as limonene which have been shown to be toxic to Tribolium castaneum [28]. Early studies on extracts of P. nigrum seeds had indicated that piperine and other active piperamides were responsible for the toxicity of the extracts to the adzuki bean weevil, Callosobruchus chinensis L. [28,29]. Piper nigrum seed oil formulations were found to be effective for protecting stored wheat from both stored grain pests, Sitophilus oryzae [L.] and Rhizopertha dominica [F.], for more than 30 days, at concentrations of 100 mg/L and higher [30]. Ethyl acetate extracts of P. nigrum seeds were also reported to be toxic to Lepidopteran and Hymenopteran herbivorous insects such as eastern tent caterpillar Malacosoma americanum F., forest tent caterpillar Malacosoma disstria Hubner, pine sawfly Diprion similis Hartig, gypsy moth Lymantria dispar [L.], spruce budworm Choristoneura fumiferana [Clemens], European pine sawfly Neodiprion sertifer Geoffroy and spindle ermine moth larvae Yponomeuta cagnagella [Hubner] [31,32].
The toxic effect of *P. nigrum* was also reported against some test insects. *Piper nigrum* was shown to be most toxic to *Callosobruchus chinensis*, *Acanthoscelides obtectus*, *C. cephalonica*, *Ephestia cautella* Hubn., followed by *Oryzaephilus surinamensis* [L.], *Sitophilus zeamais* Mosteh., *Rhyzopertha dominica* [Fab.] and *Tribolium castaneum* Herbst [Ponce de Leon, 1983]. The high toxicity effects of *P. nigrum* extracts against *C. cephalonica* larvae are attributed to the presence of high concentrations of well-known toxic components such as caryophyllene and piperine.

In the case of *Jatropha curcas*, it is a multipurpose plant with many properties and considerable insecticidal potential [33]. Different parts of *J. curcas* contain the curcin and phorbol ester which are toxic alkaloids that inhibit animals from feeding on it [34]. The insecticidal and inhibition of progeny emergence activities of oil extracted from seeds of *J. curcas* has been reported by earlier researchers against several insect pests [26,35–46]. This study is agreement with earlier studies, the petroleum ether extract of *J. curcas* seeds showed insecticidal activity against *C. cephalonica* larvae. These effects are attributed to the presence of oleic and linoleic acids which are well known toxic components.

The LC₅₀ values of petroleum ether extract of *J. curcas* seeds at 72 hours after exposure against *C. cephalonica* larvae were 13.2µL/mL [37]. Investigated toxicity of petroleum ether extracts of three different sources of *J. curcas* seeds, and noted LC₅₀ values of 8.0, 3.1 and 24.4 g/L against *S. oryzae*, respectively. [46] Studied larvicidal activity of crude methanol leaf extracts from *J. curcas* and noted LC₅₀ of 92.1 ppm against 3rd instar larvae of *Anopheles arabiensis*. In similar study, [45] reported high toxic activity of methanol leaf extracts of *J. curcas* against *Culex quinquefasciatus* Say from first to fourth instar larvae at dose levels of 1.2, 1.3, 1.4 and 1.5%. [47] Reported toxic effects of petroleum ether extracts of *J. curcas* against larvae of *Culex quinquefasciatus* Say [LC₅₀=11.3 ppm] and *Aedes aegypti* [LC₅₀=8.8 ppm]. The petroleum ether extracts of *J. curcas* were reported to be more efficient than other tested plant extracts. Mortality percent also was highly significant for petroleum ether extract of *P. nigrum* and *J. curcas*. According to Chauhan the extracts of *Croton sparsiflorus* [LC₅₀=0.073], *Anona squamosa* [LC₅₀=0.278] and *Acorus calamus* [LC₅₀=1.072] showed potential as safe insecticide. Pathak and Tiwari reported at 0.25% dose level of neem leaf larval mortality 17 ± 1.78%, while 100% mortality they reported at 3.5% dose level of neem leaf. Jadav reported LC₅₀ values of *Annona squamosa* [14.36], *Tephrosia purpurea* [38.05] and *Acorus calamus* [33.11] after 72 h. Some possible reasons for these differences are insect strains, test conditions or test material. Larval mortality was increased with the increase of plant extracts concentrations. These effects are attributed to some well known toxic compounds such as piperine, caryophyllene, limonene, α-pine, and β-pine in *P. nigrum*, and oleic acid and linoleic acid in *Jatropha curcas*.

According to many authors, any substance that reduces food consumption by an insect can be considered as an antifeedant. Isman [18] defined antifeedants as behavior-modifying substances that deter through a direct action on taste organs in insects. This definition excludes chemicals that suppress feeding by acting on the central nervous system, or a substance that has sublethal toxicity to the insect. Feeding inhibition in insect pests is the most important in the search for new and safer methods for pest control in stored grains. The high antifeedant effects of *P. nigrum* powder against *Callosobruchus maculatus* at 25 and 30 g/kg on black gram were reported by [17]. They attributed the antifeedent properties of *P. nigrum* to the piperine that killed the beetles earlier. Pepper seed extracts had also been shown to detered Lily leaf beetles *Lilicoris lilii* Scopoli and *Acalyymna vittatum* from damaging leaves of lily and cucumber plants respectively at concentrations in the 0.1-0.5 range [9]. The finding of the present study are also is in agreement with that of [40] who reported good protection of cowpea seeds from *Callosobruchus maculatus* damage in storage due to use of *J. curcas* seed oil as a repellent and antifeedant. They also reported doses of 1.0 mL/150 g grains and above gave superior mortality of the pest in cowpea. The mosquitocidal assay against fourth instar *Aedes aegypti* larvae showed that both linoleic and oleic acids in *P. nigrum* and *J. curcas* towards *Tribolium castaneum* larvae are likely to be the main cause of growth inhibition (Table 2). Both plant extract showed harmful effect on *C. cephalonica* larvae growth and development.

Significant reductions in egg hatchability revealed the harmful effects of petroleum ether extract of *P. nigrum* and *J. curcas* towards *C. cephalonica* eggs. This observation is in agreement with that of [25] who reported that food treated with *Jatropha gossypifolia* seed extract strongly inhibit the fecundity of *Tribolium castaneum* compared with *Tribolium confusum* at doses of 8000 and 16000 ppm.

These results are attributed to the physico-chemical action of the

**Table 3:** Effect of petroleum ether extracts of *Piper nigrum* and *Jatropha curcas* on egg hatchability in *Corcyra cephalonica*.

| Extract                        | Dosage (µL/mL) | Egg hatching % (Mean ± SEM)* |
|-------------------------------|---------------|------------------------------|
| Petroleum ether extract of *P. nigrum* |               |                              |
| Control                       | 93 ± 2.55 a   |                              |
| 2                             | 59 ± 4.09 b   |                              |
| 4                             | 49 ± 1.87 bc  |                              |
| 6                             | 27 ± 2.55 e   |                              |
| 8                             | 22 ± 7.00 ef  |                              |
| 10                            | 9 ± 1.92 f    |                              |
| Petroleum ether extract of *J. curcas* |           |                              |
| Control                       | 91 ± 2.92 a   |                              |
| 2                             | 58 ± 2.55 bc  |                              |
| 4                             | 48 ± 3.39 bc  |                              |
| 6                             | 32 ± 2.55 de  |                              |
| 8                             | 20 ± 2.24 ef  |                              |
| 10                            | 8 ± 1.22 f    |                              |

*Data are average of 5 replicates. Means within columns followed by the same letters are not significantly different (P<0.05; Tukey’s multiple comparison test)*

**Table 4:** *Corcyra cephalonica* adult emergence from rice kernels treated with petroleum ether extracts of *Piper nigrum* and *Jatropha curcas*.

| Extract                        | Dosage (µL/mL) | Adult emergence % (Mean ± SEM)* |
|-------------------------------|---------------|--------------------------------|
| Petroleum ether extract of *P. nigrum* |               |                               |
| Control                       | 86 ± 1.87 a   |                               |
| 2                             | 3 ± 1.22 bc   |                               |
| 4                             | 1 ± 1.00 bc   |                               |
| 6                             | 0 ± 0.00 c    |                               |
| 8                             | 0 ± 0.00 c    |                               |
| 10                            | 0 ± 0.00 c    |                               |
| Petroleum ether extract of *J. curcas* |            |                               |
| Control                       | 85 ± 2.74 a   |                               |
| 2                             | 8 ± 1.22 b    |                               |
| 4                             | 5 ± 1.58 bc   |                               |
| 6                             | 3 ± 2.00 bc   |                               |
| 8                             | 2 ± 1.22 bc   |                               |
| 10                            | 0 ± 0.00 c    |                               |

* Data are average of 5 replicates. Means within columns followed by the same letters are not significantly different (P<0.05; Tukey’s multiple comparison test)
components including piperine, caryophyllene, limonene, oleic acid, linoleic acid, menthone, menthol, α-pinene and β-pinene. Inhibition in egg hatching of the pulse beetle, Callosobruchus chinensis with P. nigrum essential oils were reported by [49], who observed that egg hatching was inhibited significantly when fumigated with sublethal concentration of the essential oil. Inhibition in adult emergence with P. nigrum was reported by [17] who observed lesser number of Callosobruchus maculatus adults emerging in black gram seeds treated with P. nigrum powder at doses of 25 and 30 g/kg. In treatments with P. nigrum oils, regression analysis showed a dose-dependent significant correlation with adult emergence [F=160.15], with the number of adults emerging from the fumigated larvae decreasing in concentration dependent manner [49]. High efficacy of the hexane extract of P. nigrum on 2nd instar larvae of Spodoptera litura was obtained with adult emergence of 19.79% for treatments of up to 40 mg/ml compared to the control [83.12%] [50].

Inhibition of progeny emergence with J. curcas extracts were studied by [38]. It was shown that water extracts of dried ground seeds of J. curcas at a dose of 5% [w/w] significantly reduced S. zeamais progeny emergence in treated grains of maize and cowpea.

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