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Evaluation of biological activities of *Piper nigrum* oil against *Tribolium castaneum*

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Abstract

The essential oil of *Piper nigrum* (L.) was evaluated for its repellent, insecticidal and developmental inhibitory activities against an important wheat grain pest *Tribolium castaneum* (Herbst). Adults of *T. castaneum* were repelled significantly by *P. nigrum* at 0.2% concentration (v:v) and above in filter paper test. The LC_{50} values for larvae and adults were calculated to be 14.022 µl and 15.262 µl respectively. The oil had shown a dose response relationship as the larval and adult mortality increased while the larval survival and adult emergence decreased with increase in the concentration of essential oil. Effective concentration (EC_{50}) of *P. nigrum* oil to reduce the number of larvae transformed to pupae to 50% was found to be 6.919 µl.

Key words: essential oil, *Piper nigrum*, *Tribolium castaneum*.

Introduction

In their search for pest control agents with limited undesirable effects on the environment and non-target organism, researchers are continuously evaluating botanical resources (Grainge *et al*., 1986), and exploring new botanicals possessing high pesticidal activity to resolve the pesticide dilemma (Berenbaum, 1989). For pest control massive use of synthetic pesticides has imposed so many detrimental effects on the environment and cause intoxication of non-targeting organisms (Farage Elawar, 1989; Markowitz, 1992; Rajeskaran and Baker, 1994; Gupta *et al*., 2001). However, these chemicals are declared ecologically unsafe because these persist for longer period in the environment and enter in to the food chain. During last few decades various synthetic pesticides have been applied to protect stored grains and other agricultural products from insect infestation. But it has been reported that certain insect pests have acquired resistance against most of the insecticides (Brattsten *et al*., 1986; Zettler and Cuperus, 1990; White, 1995). Thus it is necessary to develop certain new safe alternatives of these synthetic pesticides, which might have no adverse effect on the environment and non-target animals.

In this direction, many plant products such as essential oils, crude extracts have been explored for their insecticidal properties against field crop (Sharma *et al*., 2001; Tare, 2001; Grover *et al*., 2000; Patel and Patel, 1999; Hashim and Devi, 2003) and stored grain pests (Malik and Naqvi, 1984; Su, 1990; Dunkel and Sears, 1998; Wongo, 1998). Moreover, essential oils of many common spices have also been evaluated for their repellent and insecticidal activities (Tripathi *et al*., 2000a; 2000b; Isman *et al*., 2000). However, some of them have been reported highly repellent against aphids *Aphis gossypii* Glover (Zhou *et al*., 2004), *Sesamia nonagrioides* Lefèbvre (Konstantopoulou *et al*., 2004), *Aedes albopictus* (Skuse) (Yang and Ma, 2005), *Musca domestica* L. (Abdel Hady *et al*., 2005), *Aphidius colemani* Viereck (Bostanian *et al*., 2005) and *Ceratitis capitata* (Wiedemann) (Hussein, 2005). Similarly volatile constituents from *Securidaca longepedunculata* Fresen (Jayasekara *et al*., 2005) and *Daucus carota* L. (Nissinen *et al*., 2005) are used to check the efficacy against stored grain pests. Beside crude oils, toxic effects of oil constituents were also established against many insect pests (Weaver *et al*., 1991; 1995; Rao and Singh, 1994).

Beside essential oils, insecticidal potential of certain plant extracts such as *Piper guineense* (Schumacher et Thonning) (Asawalam *et al*., 2007), *Piper nigrum* (L) (Boff *et al*., 2006), *Anethum graveolens* L., *Cuminum cyminum* L. (El Lakwah *et al*., 2002), *Allium sativum* L. (Udo, 2005), *Vitex negundo* L., *Polygonum hydropiper* L. (Taleb and Salam, 2005), *Myristica fragrans* Houttuyn (Haryadi and Rahayu, 2003) has been established against stored grain pests (Trakoontivakorn *et al*., 2006). In the present study, insecticidal, repellent and development inhibition activity of *P. nigrum* oil have been reported against wheat grain pest *Tribolium castaneum* (Herbst) (Coleoptera Tenebrionidae) to control its infestation.

Materials and methods

Isolation of oil

The concerned spice *P. nigrum* (Black pepper) was purchased from the local market. The spice was grounded by domestic mixer and the powdered material was hydro-distilled in Clevenger apparatus continuously for 5 hrs to yield essential oil.

Insects

Rust red flour beetle *T. castaneum* were used to examine the activity of essential oils. For activity testing, adult and larval stages of insect were taken from laboratory rearing culture maintained at 28±2 °C, 75±5 RH and a photoperiod of 12:12 (L:D). The insects were reared on wheat flour.

Repellency

Repellency experiments were carried out in 80 mm glass Petri dish. Test solutions were prepared by dissolving different concentrations (1, 2, 3 and 4 µl) of *P. nigrum* oil in 1 ml acetone. Whatman no. 1 filter paper was cut into two and each solution was applied to half of a filter paper as uniform as possible by using micro pipette. The other half of the filter paper was treated with acetone alone. The oil treated and acetone treated halves were dried to evaporate the solvent completely. Treated and untreated halves were attached with cellophane tape and placed in the glass Petri dish. Twenty adult flour beetles (4-6 days old) were released at the centre of the filter paper disc and then sealed tightly. Six replicates were set for each concentration. Observation of the number of insects present on both the treated and untreated halves was recorded after four hours of experiment setting. For calculation insect were finally counted as repelled and unrepelled.

Larval mortality

For evaluation of larvicidal activity newly molted 4^{th} instar larvae of *T. castaneum* were exposed to various quantities of essential oil. Ten larvae taken from laboratory culture were placed with 4 g. of wheat flour in 80 mm Petri dish. The filter paper strips (1 cm^2) one in each Petri dish treated with different concentration of *P. nigrum* oil (8, 12, 16 and 20 µl) was pasted on the inner surface of the cover and finally Petri dish were made airtight. Each treatment was replicated six times. After 24 h of fumigation in darkness, larval mortality was recorded.

Adult mortality

The toxic effect was tested on well-fed adults. Ten adult insects were taken from laboratory culture and placed with 4 g of wheat flour in 80 mm Petri dish. The filter paper strips (1 cm^2) one in each Petri dish treated with different concentration of *P. nigrum* oil (8, 12, 16 and 20 µl) was pasted on the inner surface of the cover and finally Petri dish were made airtight. Each treatment was repeated six times. After 24 h of essential oil exposure in darkness, adult mortality was recorded.

Developmental inhibition

Developmental inhibition potential of *P. nigrum* was carried out against 4^{th} instar larvae of red flour beetle in the laboratory. For this ten larvae were exposed to different concentrations of essential oil (2, 4, 6 and 8 µl) in 80 mm glass Petri dish for 24 h. Treated/exposed larvae were transferred to fresh Petri dish containing flour material. For each concentration six replicates were set for test and control to evaluate developmental inhibition. Number of larvae survived, pupae transformed from survived larvae and adult emerged from transformed pupae were recorded. For developmental inhibition insects were regularly observed for 40 days.

Data analysis

Chi-square test was applied to establish the repellent activity of the oil tested (Sokal and Rohlf, 1973)· Linear regression analysis was performed to show all dose-response relationship (Sokal and Rohlf, 1973). Analysis of variance (ANOVA) was performed to test the equality of regression coefficients (Sokal and Rohlf, 1973). The LC_{50} and EC_{50} values were calculated by POLO programme (Russel *et al.*, 1977).

Results

Repellency

Chi-square test analysis indicated that the *P. nigrum* oil tested was repellent to *T. castaneum*. It had shown significant repellent activity at 0.2 percent concentration and above as the hypothesis of ratio 1:1 was rejected (table 1).

Larvicidal and adult mortality

The *P. nigrum* oil tested killed the 4^{th} instar larvae and adults by vapor action. The LC_{50} value to kill the half of the larval and adult population was found to be 14.022 µl and 15.262 µl (table 2). Regression analysis showed a dose dependent significant correlation of the oil exposure with larval mortality (F=175.281 at df 4 and 25, table 3) and adult mortality (F=160.892 at df 4 and 25, table 3).

Developmental inhibition

The percentage of larvae that reached the pupae and the percentage of pupae that reached the adult stage decreased with an increase in concentration of *P. nigrum* essential oil (figure 1). The EC_{50} value that reduced the number of larvae transformed to pupae to 50% were found to be 6.919 µl (table 2). Regression analysis showed a dose dependent significant correlation of the oil exposure with larval survival (F=21.428 at df 4 and 25), pupal survival (F=60.123 at df 4 and 25) and adult emergence (F=160.150 at df 4 and 25) (table 3, figure 1).

Table 1. Repellency caused by *P. nigrum* oil towards adult *T. castaneum* after 4 h in the filter paper test.

Concentration of oil used in µl	Mean no. of insects repelled	Mean no. of insects unrepelled	Expected value of insect repelled	Expected value of insect unrepelled	χ^2 value P<0.5 (df=5)	EC_{50}
0.1	11.66	8.33	10	10	0.456 NS[a]	0.071
0.2	17.00	3.00	10	10	0.0017 NS[a]	
0.3	19.00	1.00	10	10	5.699 S[b]	
0.4	19.60	0.66	10	10	2.280 S[b]	

Adults of *T. castaneum* were used for repellency experiment in the filter paper test. For each concentration of oil, six replicates were taken and in each replicate 20 adult insects were used.

a. Not significant (NS) as the calculated values of χ^2 were less than the table values at all probability levels (90%, 95% and 99%).

b. Significant (S) at all probability levels (90%, 95% and 99%).

Table 2. Summary of the toxicity assays.

Parameters	LC_{50}/EC_{50} [a] at P<0.05	LCL[b]	UCL[b]	g-value[c]	t-ratio[c]	heterogenity[c]
1. Larval mortality	14.022 µl	12.839 µl	15.383 µl	0.074	7.217	0.31
2. Adult mortality	15.262 µl	13.708 µl	17.445 µl	0.110	5.899	0.27
3. Larval survival	6.919 µl	5.809 µl	9.072 µl	0.125	5.546	0.40

a. LC_{50} and EC_{50} represent lethal concentrations that cause 50% mortality and 50% reduction in survival of *T. castaneum* respectively.

b. LCL and UCL mean lower confidence limit and upper confidence limit respectively.

c. g value, t ratio and heterogeneity were significant at all probability levels (90%, 95% and 99%).

Table 3. Regression parameters of insecticidal and developmental inhibition effects of *P. nigrum* oil against *T. castaneum*.

Parameters	Intercept	Slope	R	F-value[a] at df 4 and 25
1. % Larval mortality	-5.002	3.541	0.9758	175.281
2. % Adult mortality	-4.912	3.474	0.9755	160.892
3. % Larval survival	80.326	-3.668	-0.8200	21.428
4. % Pupal survival	111.336	-6.168	-0.9819	60.123
5. %Adult emergence	142.338	-20.835	-0.9997	160.150

Regression was performed between dose of *P. nigrum* oil and responses of *T. castaneum*.

a. F- values were significant at all probability level (90%, 95% and 99%).

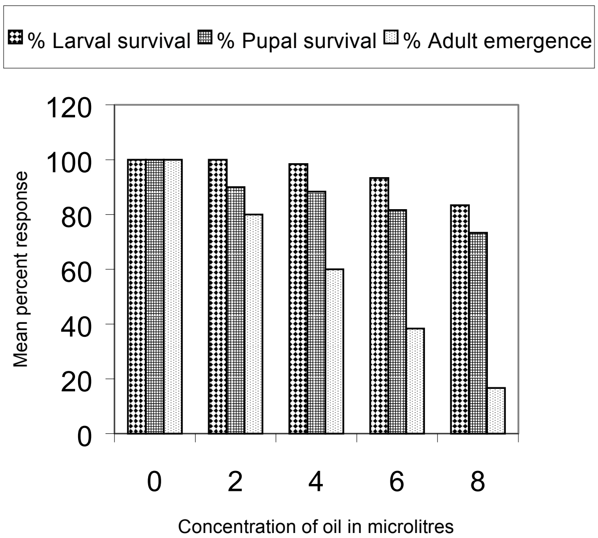

Figure 1. Mean percent of larval survival, pupal survival and adult emergence after exposure to different concentrations of *P. nigrum* oil.

Discussion

For management of insect pests many plant products such as essential oils and solvent extracts have been screened for their repellent, antifeedant, toxic and growth inhibitory activities against stored grain pests (Maliq and Naqvi, 1984; Matthews, 1993). In the present investigation adults of *T. castaneum* were found to be significantly repelled at concentration of 0.2 µl of *P. nigrum* oil in comparison to controls. The chi-square values was found only non specific 4.40 at 0.1 µl concentrations, but rest of the concentration were found significant at p<0.05 level (table 2). Further regression analysis shows insecticidal and developmental inhibition of *T. castaneum* as oil has suppressed the percent survival of both larvae and pupa (Slope value –3.668 and –6.168) and heavily cut down % adult emergence (slope value –20.835) (table 3). Similarly volatile oils isolated from *Chenopodium ambrosiodes* L., *Thymus vulgaris* L. and have shown insecticidal activities against *Lucilia sericata* (Meigen) (Morsy *et al*., 1998) while *Satureja hortensis* L. against *Spodoptera litura* (F.) larvae (Isman *et al*., 2000). Volatile compound diallyl disulphide isolated from neem has shown potent toxic, fumigant and feeding deterrent activity against stored grain pests i.e. *Sitophilus oryzae* (L.) and *T. castaneum* (Koul, 2004).

Essential oils obtained from eucalyptus, margoram, pennyroyal and rosmary have shown insecticidal activity against *Pediculus humanus capitis* De Geer (Yang *et al*., 2004). *S. longepedunculata* root powder, its methanol extract and volatile component exhibited repellent and toxic properties against *Sitophilus zeamais* Motschulsky adults (Jayasekara *et al*., 2005). The LC_{50} values were 34-36 µl for stored grain pests. Cinnamaldehyde isolated from *Cinnamomum osmophloeum* Kanehira has shown larvicidal activity against *Aedes aegypti* (L.) (Cheng *et al*., 2004). Besides this, both cinnamaldehyde and cinnamyl acetate have shown excellent inhibitory activity against fourth instar larvae of *A. aegypti,* LC_{50} value was 29 ppm. Eugenol and methyl salicylate essential oils isolated from leaf buds of *Eugenia caryophyllata* Thunberg have shown significant toxicity potential against *P. capitis* (Yang *et al*., 2003).

Besides above reports essential oils isolated from

Ipomoea cairica (L.) oil has shown larvicidal effect against *Culex tritaeniorhynchus* Giles (100 ppm), *A. aegypti* (120 ppm), *Anopheles stephensi* Liston (120 ppm) and *Culex quinquefasciatus* Say (170 ppm) (Thomas *et al*., 2004), while some African plants have shown fumigant toxicity against *Anopheles gambiae* Giles (Omolo *et al*., 2005). Essential oils from mentha, carvacryl, citronella and eucalyptus have shown good repellent effects against *A. albopictus* (Yang and Ma, 2005), while *Artemisia princeps* Pamp and *Cinnamomum camphora* (L) oils have shown repellent and insecticidal activity against *S. oryzae* and *Bruchus rufimanus* Bohemann (Liu *et al*., 2006). Beside this, volatile oils isolated from leaves and flowers of *Lantana camara* L., *Callistemon lanceolatus* DC., *Cymbopogon winterianus* Jowitt, *Eucalyptus* sp., *Nerium oleander* L., *Ocimum basilicum* L., *Ocimum sanctum* L. and *V. negundo* (Sharma *et al*., 2001), *S. hortensis*, *Thymus serphyllum* L. and *Origanum* sp. (Isman *et al*., 2000) have shown insecticidal, antifeedant and growth inhibitory activities against insect pests.

The results obtained from the present study demonstrated that *P. nigrum* essential oil is highly repellent and toxic to adults and growing larvae of *T. castaneum*. It repelled the beetles significantly even at very low concentration. The oil also inhibits development of larvae into pupae and pupae into adult significantly. The steep slope values indicate that even small increase in the concentration cause a high mortality and developmental inhibition in the pest. Values of the t ratio greater than 1.96 indicate that the regression is significant. Values of the heterogeneity factor less than 1.0 denote that in the replicate test of random samples, the concentration response lines would fall within 95% confidence limits and thus the model fits the data adequately. The index of significance of potency estimation, g value, indicates that the value of the mean is within the limits at all probabilities (90, 95, 99) as it is less than 0.5.

In the present investigation *P. nigrum* essential oil is identified most effective against *T. castaneum* infestation. It has shown toxic, repellent and development inhibition activity to the larvae and pupae of the *T. castaneum*. Therefore it can be potentially used for disruption of larval and pupal development. Our findings clearly indicate that the *P. nigrum* oil can work as a promising natural pesticide, which can effectively reduce the beetle population in godowns. Further studies, at constituent level along with structure activity will be required to explore the broad-spectrum biological activity of this essential oil.

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