The biological effects of the water extract of *Piper nigrum* (Fam: Piperaceae) seeds on the larvae of *Sarcophaga haemorrhoidalis* (Fallen) (Diptera: Sarcophagidae)

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Abstract: The water extract of *Piper nigrum* was evaluated in the laboratory against the second and third larval instar of the flesh fly *Sarcophaga haemorrhoidalis*. The water extract of *P. nigrum* were tested to study their efficacy on larval mortality, larval weight, larval duration, pupation, pupal weight, adult emergence. The second larval instar was the most affected by the water extract of *P. nigrum* than the third larval instar. The water extract of *P. nigrum* showed a high level of biological activity against the second larval instar which fed on treated diet. It is induced prolongation in the larval duration, great reduction in larval weight and the larval mortality increased.
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1. Introduction:

Insect pests have been controlled with synthetic insecticide over 50 years but problems of pesticide resistance and negative effects on non-target organisms, including human and environment have been reported (FAO, Pesticide residue in food, Rome, 1992). The synthetic insecticides are more hazardous to handle, leave toxic residues in food products and are not easily biodegradable. The world flora has a variety of plant species and in order to increase the number of plants used for pest control, more studies are being carried out to identify variety of effective substances found in different plant species. Consequently, substances alternative of chemical pesticides, which pollute our natural sources and threaten our future, can be discovered. More than 2000 plant species have been known to produce chemical factors and metabolites of values in pest control programs (Sukumar *et al*., 1991).

Moursy (1997) studied The insecticidal activity of LD_{50} values, of acetone, ethanol, petroleum ether and water extracts of *Calotropis procera* leaves against the flesh fly, *Sarcophaga haemorrhoidalis* and he found that the ethanol extract of *C. procera* was the most toxic, of all solvents used, to different stages of *S. haemorrhoidalis*. Findings suggest also that *C. procera* extracts may produce larvicidal, pupicidal and adulticidal effects, (behaving like general toxicants) against the flesh fly, *S. haemorrhoidalis.*

These plant species belonging to 189 plant families which are said to be rich sources of bioactive organic compounds (Rao *et al*., 2005). Among the families of plants investigated to date, one showing enormous potential is the pepper family, otherwise known as *Piperaceae* (Dodson *et al*., 2000). The phytochemical screening of black pepper fruit shows that it contains 4% alkaloids in the berry (Dev & Koul, 1997; Awoyinka *et al*., 2006). Although information on the compounds responsible for the insecticidal activities is scarce, it has been documented that the amide olefinic or alkyl isobutylamides compounds such as piperine, piperettine, tricostacine, peepuloidin, piplartin and trichonine contribute in no small measure (Adgeh, 1989; Awoyinka *et al*., 2006). These compounds have been demonstrated to be toxic to fruit flies, adzuki bean weevils, cockroaches and several other insect species (Su & Hovart, 1981; Gbenwonyo *et al*., 1993; Awoyinka *et al*., 2006).

Sarcophagidae flies, commonly known as flesh flies, comprise 2.600 described species worldwide (Pape *et al*., 2009). Out of these, 117 species under 38 genera of three subfamilies have been described from India (Nandi, 2002; Sinha and Nandi, 2002 a, b). *Sarcophaga haemorrhoidalis*, also known as the red-tailed flesh fly, is a fly in the Sarcophagidae family. *Sarcophaga haemorrhoidalis* is a common species of medical importance in many parts of the world. Therefore, it is possible that mechanical transfer of potential disease causing pathogens, such as bacteria, viruses, protozoa, and helminth eggs, to human food may occur (Greenberg 1973; Sukontason, *et al*., 2000). Larvae of this species are known to cause myiasis in several mammal species, including humans (Zumpt, 1965; Kumarasinghe, *et al*., 2000). Another facet of medical importance of this fly is its association with human corpses and its relevance to forensic entomology, *S. haemorrhoidalis* were found connected with cases of human death (Lee 1996; Carvalho, *et al*., 2000; Goff 2000; Sukontason, *et al*., 2010). This fly often breeds in carrion and feces, making it a possible vector for disease (Allotey, 2011).

The larvae of this species can cause myiasis, as well as accidental myiasis. Due to its attraction to feces

and carrion, *S. haemorrhoidalis* has been accounted for as a dipteran species that may serve as a mechanical vector for disease, especially if it intrudes homes. The larvae of *S. haemorrhoidalis* may produce myiasis on necrotic or dead flesh. Larvae of flesh flies breed in carrion, faeces or decaying organic matter (Amoudi *et al*., 1992; Al-Misned 2000; Al-Misned *et al*., 2001). Some species of Sarcophaginae are predatory on small invertebrates (Pape 1987; 1996 Mendez and Pape 2002). It has also been reported that their larvae can cause traumatic human myiasis (Zumpt 1965; Colwell and O'Connor 2000).

Blow flies and flesh flies are used to estimate the length of post-mortem interval and provide evidence in criminal investigations (Joyce, 1984; Greenberg, 1985; Smith, 1986; Anderson 1997; Amendt, *et al.*, 2000).

Adhikari and Chandra (2014) analyzed the larvicidal, smoke toxicity, repellency and adult emergence inhibition activity of crude and solvent extracts of *Swietenia mahagoni* leaves against larva of *Anopheles stephensi* and they found about 97% mortality of third instars larvae was found in 0.5% crude and 80 mg/L ethyl acetate extracts of mature leaves after 72 h of exposure, They reported that the leaf extracts of *S. mahagoni* showed remarkable effect as larvicide, smoke toxic, repellent and adult emergence inhibitor against *A. stephensi*.

Hence, the present study was conducted to evaluate the biological effects of *P. nigrum* extracts on the2 nd and 3rd larval instar. of *S. haemorrhoidalis*.

2.Material and Methods:

1- Plant collection and extraction:

The plant chosen for this study namely *Piper nigrum*, the seeds of this plant were brought from the market. The method of plant extraction was modified from those of Satoto (1993) and Choochote *et al*., (1999). Five hundred grams of seeds of *Piper nigrum* was ground and filtered using a strainer silver number 60. The powder was macerated with 1.5 L of 80% ethanol solution and left to stand at room temperature for 3 days. The mixture was filtered through a Whatman no.1 filter paper by suction and the filtrate was evaporated under vacuum at 40° C until completely dried, the crude extract was kept in refrigerator until needed for tests.

2- Rearing technique and bioassays:

The stock colony of *S. haemorrhoidalis* was set up in the laboratory starting from flies collected from the north of Jeddah city. The colony was reared successfully under laboratory conditions of 25 ± 2° C. and 70 ± 5 % R.H. Two-day-old (2 nd larval instar) and three-day-old (3 rd larval instar) were collected and used for bioassay tests. The tested plant extract was evaluated by mixing 10 ml of different concentrations (1, 3 and 5%) with the larval medium ground meat. Control experiments were carried out using tap water only. The larvae were fed continuously until pupation. Each experiment was replicated three times and each replicate 50 larvae were used. Percent larval mortality, larval duration and larval weight gain were calculated.

3- Calculations and data analysis:

The percent larval mortality was corrected according to Abbott ' s formula (1987): $\frac{X-Y}{X}$ x100 = percent corrected mortality

Where: X = the percent living in the check (control).

Y= the percent living in the treated.

The reduction of larval and pupal weight, pupation and adult emergence were calculated according to Khazani (1979):

$$\% \text{ Reduction} = \frac{C-T}{C} \text{ x100}$$

Where: C = the number of insects in the control.

T = the number of insects in the treatment.

Statistical analysis of the data was carried out according to the method of

Lentner *et al*., (1982).

3.Results:

1- Larval mortality:

Data given in table (1) indicate that the second larval instar was the most affected in larval mortality than that of the third instar after treatment with the water extract of *Piper nigrum*. The second larval instar induced 20.8, 54.2 and 83.3 % larval mortality at concentrations 1, 3, and 5 % respectively. While the third instar induced 2.1, 4.2 and 8.3 % larval mortality at concent-rations 1, 3, and 5 % respectively (Fig. 1).

2- Larval weight:

Results presented in table (1) show that the second larval instar was the most affected in larval weight than the third larval instar. The rate of reduction was highly increasing the concentration of the extract in the larval diet, where the initial weight decreased by 5.3 at 1% 12.1% at 3% and 28.5 at 5% in the second larval instar, while the third larval instar induced a decrease in weight gain of larvae. The percent weight gain decreased by increasing the concentration of the extract in the larval diet (Fig. 2).

3- Larval duration:

Data obtained in table (1) showed that the water extract of *P. nigrum* treatment caused a significant prolongation in the larval period if the second larval instar compared with the third instar. The larval duration was 7.0, 7.8 and 8.8 days at concentrations 1, 3 and 5% respectively as compared to 6.4 days for the control. The larval duration was not affected in the treatment of the third instar (Fig. 3).

4- Pupation:

Results in table (2) show that the second larval instar was the most affected on percent pupation than the third instar. The second instar elicited 2.1, 4.2 and 8.3 % reduction in pupation, while the second instar induced 22.8, 52.2, and 80.4% reduction in pupation at concentrations 1, 3 and 5% respectively, while the third instar induced 4.1, 6,2 and 6.1 % at concentrations 1, 3 and 5% respectively (Fig. 4).

5- Pupal weight:

From data presented in table (2), it is concluded that the water extract of *P. nigrum* slightly affected the pupal weight when the treatment of the third instar, as the reduction percentages in pupal weight were 1.2, 4.9 and 7.4% at concentrations 1, 3 and 5% respectively, while the second instar was the most affected and induced 13.5, 25.9 and 41.9 % at concentrations 1, 3 and 5% respectively (Fig. 5).

6- Pupal duration:

The pupal period increased when we apply the treatment on the second instar, while the pupal period was not affected with the treatment of the third larval instar with the water extract of *P. nigrum*. The pupal duration when we apply the treatment on the second instar was 5.1, 5.4 and 5.7 days at concentrations 1, 3 and 5% respectively as compared to 4.5 days for the control (table 2- Fig. 6).

7- Adult emergence:

The water extract of the tested plant induced reduction in adult emergence, as the reduction percentages in adult emergence when we apply the treatment on the second larval instar were 30.3, 62.5 and 84.4 % and with the third larval instar were 11.7, 14.5 and 21.9 % at concentrations 1, 3 and 5% respectively (Table 2. Fig. 7).

Table (1): Effect of water extract of *Piper nigrum* on larval mortality, larval weight and larval duration of 2nd and 3rd larval instar of *Sarcophaga haemorrhoidalis*.

Larval instar	Con.(%)	Larval weight			% Corrected larval mortality	Larval duration in (days ± S.E.)
		Mean weight at the beginning (mg) S.E.	Mean weight after 2 days (mg) S.E.	% weight gain (+) or less (-)		
Control	-	5.2 ±0.1	7.2± 0.1	48.6	-	6.4±0.1
Second larval instar	1	5.6 ± 0.3	5.4± 0.3	5.3 (-)	20.8	7.0±0.3
	3	5.5 ± 0.1	5.0 ±0.2	12.1 (-)	54.2	7.8±0.3
	5	4.7 ± 0.3	6.7 ±0.3	28.5 (-)	83.3	8.8±0.4
F value	-	-	-	-	-	37.51*
Third larval instar	1	5.3 ±0.1	7.3± 0.3	47.5	2.1	6.4 ±0.1
	3	5.5 ± 0.3	7.2 ±0.3	38.8	4.2	6.5± 0.1
	5	6.0 ±0.2	6.7 ±0.3	15	8.3	6.6±0.2
F value	-	-	-	-	-	1.71 NS

NS: Non significant.

*: significant at 0.05 level of probability (F=7.59).

Table (2): Effect of water extract of *Piper nigrum* on pupation, pupal duration, pupal weight and adult emergence of 2nd and 3rd larval instar of *Sarcophaga haemorrhoidalis*.

Larval instar	Conc. (%)	Pupation (%)	Reduction in pupation (%)	Pupal duration in (days ± S.E.)	Pupal weight (mg)	Reduction in pupal weight (%)	Adult emergence (%)	Reduction in adult emergence (%)
Control	-	98	-	4.5 ± 0.2	9.1 ± 0.1	-	97	-
Second larval instar	1	76	22.8	5.1± 0.3	8.0 ± 0.2	13.5	67.4	30.3
	3	46	52.2	5.4 ± 0.3	7.0± 0.3	25.9	35.6	62.5
	5	18	80.4	5.7 ± 0.3	5.7± 0.3	41.9	13.3	84.4
F value	-	-	-	11.70 *	399*	-	-	-
Third larval instar	1	94	4.1	4.4 ± 0.3	9.0 ± 0.3	1.2	86.6	11.7
	3	92	6.2	4,3 ± 0.2	8.7 ±0.2	4.9	83.4	14.5
	5	88	6.1	4.4 ± 0.3	8.5 ± 0.3	7.4	76.5	21.9
F value	-	-	-	0.09 NS	5.41 NS	-	-	-

NS: Non significant.

*: significant at 0.05 level of probability (F=7.59).

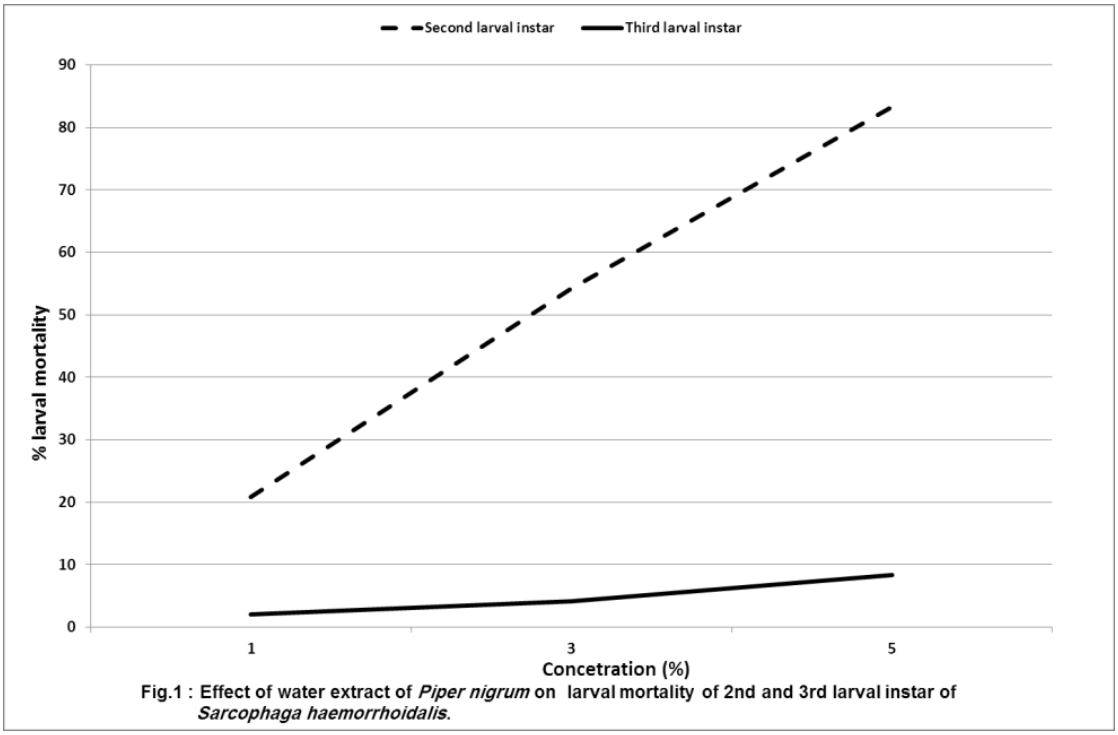

Fig.1 : Effect of water extract of *Piper nigrum* on larval mortality of 2nd and 3rd larval instar of *Sarcophaga haemorrhoidalis*.

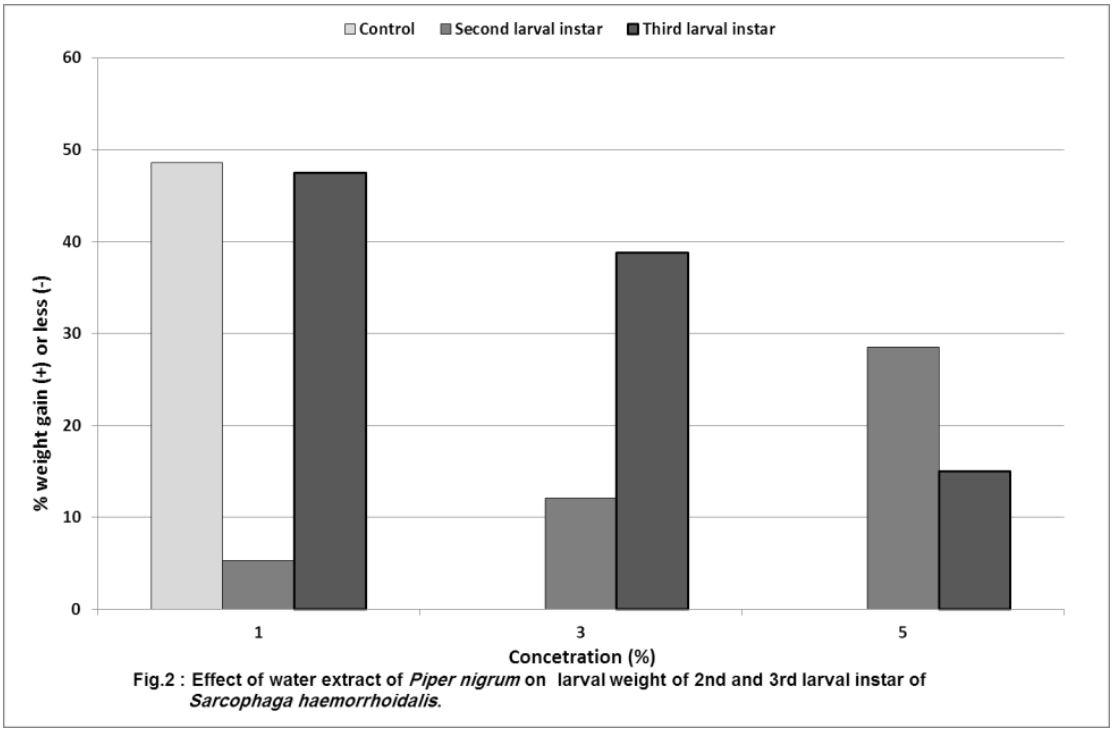

Fig.2 : Effect of water extract of *Piper nigrum* on larval weight of 2nd and 3rd larval instar of *Sarcophaga haemorrhoidalis*.

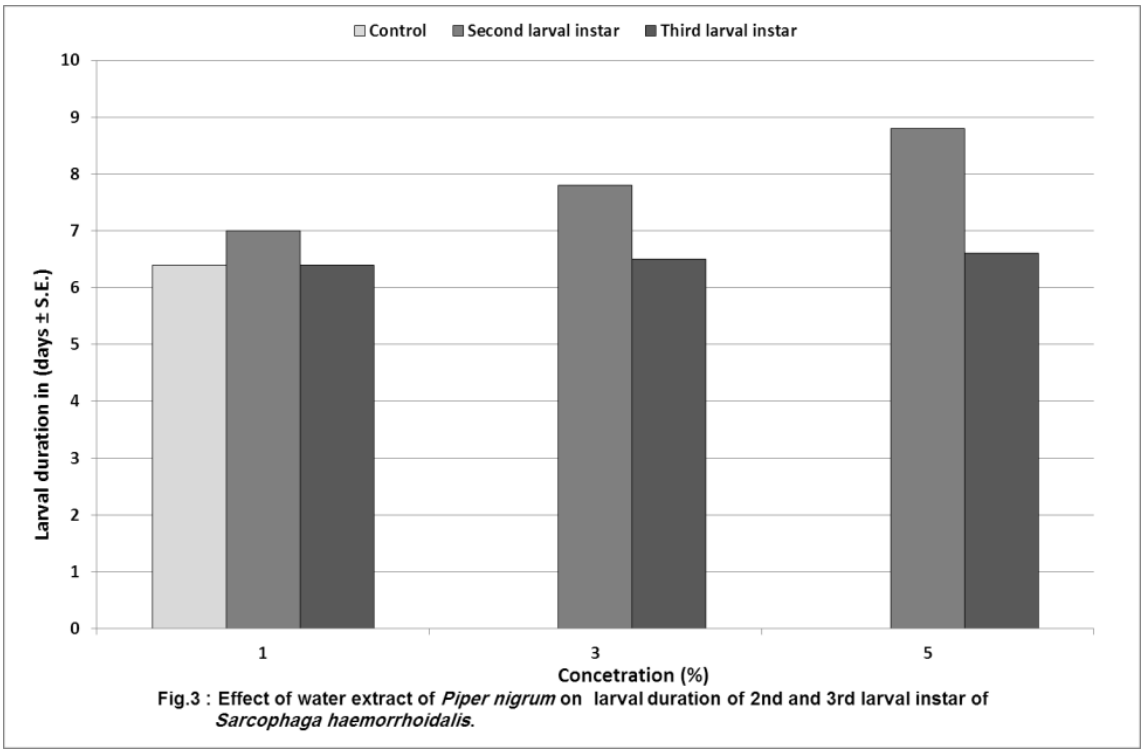

Fig.3 : Effect of water extract of *Piper nigrum* on larval duration of 2nd and 3rd larval instar of *Sarcophaga haemorrhoidalis*.

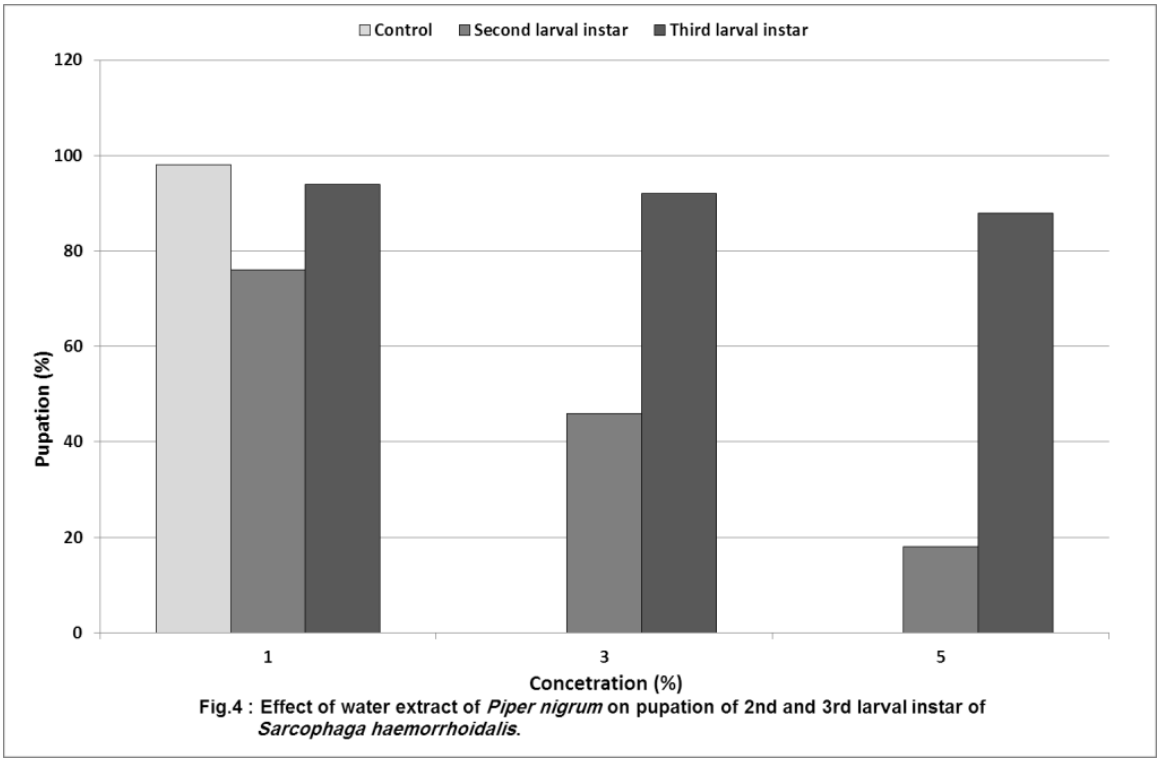

Fig.4 : Effect of water extract of *Piper nigrum* on pupation of 2nd and 3rd larval instar of *Sarcophaga haemorrhoidalis*.

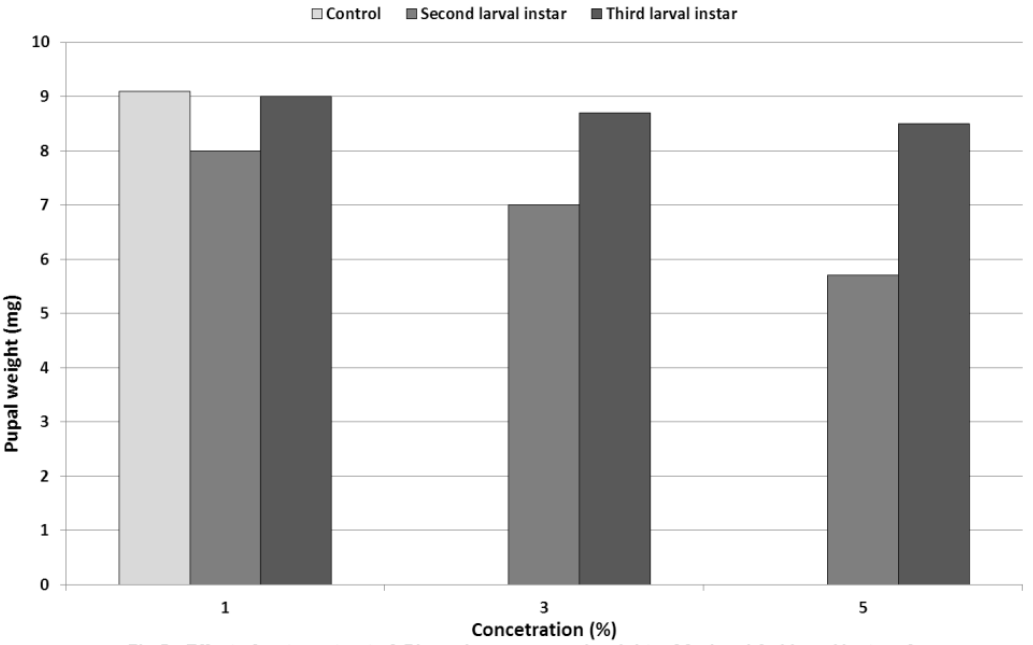

Fig.5 : Effect of water extract of *Piper nigrum* on pupal weight of 2nd and 3rd larval instar of *Sarcophaga haemorrhoidalis*.

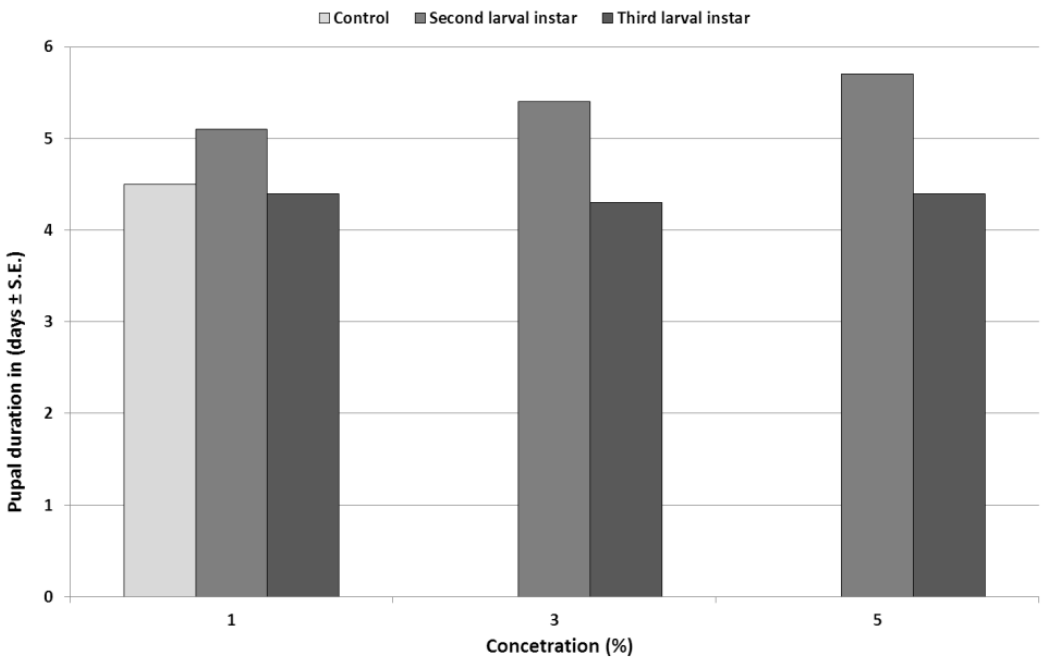

Fig.6 : Effect of water extract of *Piper nigrum* on pupal duration of 2nd and 3rd larval instar of *Sarcophaga haemorrhoidalis*.

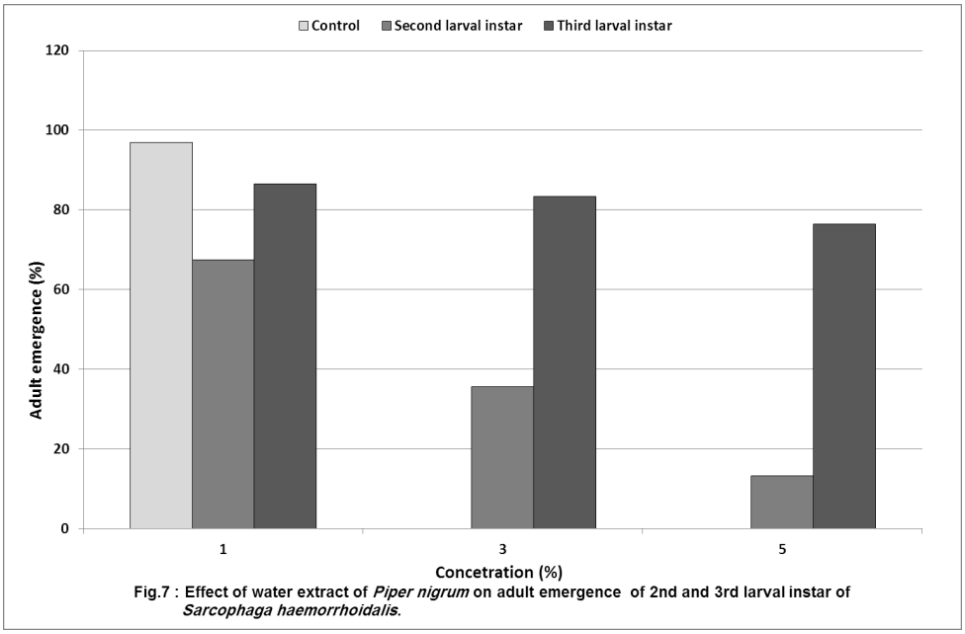

Fig.7 : Effect of water extract of *Piper nigrum* on adult emergence of 2nd and 3rd larval instar of *Sarcophaga haemorrhoidalis*.

4.Discussion:

Today, the environmental safety of an insecticide is considered to be of paramount importance. An insecticide does not have to cause high mortality on target organisms in order to be acceptable (Kabaru and Gichia, 2001) but it should prevent the breeding. Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive, and are readily available throughout of the world. According to Bowers *et al.*, (1995) the screening of locally available medicinal plants for pests control would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health. The biological activity of these plant extracts might be due to the various compounds, including phenolics, terpenoids, and alkaloids, existing in plants (Park *et al.*, 2000). These compounds may jointly or independently contribute to produce larvicidal activity against *S. haemorrhoidalis.*

The present study revealed that the water extract of *P. nigrum* significantly affected the larval mortality, larval weight gain, larval duration, pupation, pupal weight, pupal duration and adult emergence of the second and third larval instar of *S. haemorrhoidalis*.

Results obtained in the present study indicated that the toxicity of plant extract against the 2nd 3rd instar larvae of S. haemorrhoidalis was extended to the adults causing mortality reached to 84.4 %. Similar observations on other plant extracts effect on several insects have been reported. For example, Gad Allah (1991) who stated that the extracts of *M. azedarach* and *V. rosea* caused prolongation in larval and pupal duration of *M. domestica*. Also, the extracts of *M. azedarach*,*V. rosea* and *A. sativum* induced reduction in pupation and adult emergence. Shalaby *et al*.,(1998) using peel oils of lemon, grapefruit and naval orange against *C. pipiens* larvae.

Zhong *et al.* (2001) have also highlighted that extract from *Rhododendron molle* flowers extend the duration of development of *Pieris rapae*. Papachristos and Stamopoulos (2002) have reported that larvae of *Acanthoscelides obtectus* were more susceptible than pupae to the fumigant toxicity of the essential oils from *Lavandula hybrid*, *Rosmarinus officinalis* and *Eucalyptus globules.* Scott *et al*., (2003) have reported that pupal stage of *Leptinotarsa decemlineata* was less sensitive to the *Piper nigrum* extracts then the larval stages. Sadek (2003) showed that the time of pupation of *Spodoptera littoralis* of larvae increased by the extract of *Adhatoda vasica.* Jeyabalan *et al*., (2003) have reported that extract of *Pelargonium citrosa* prolonged the duration of larval instars and the total developmental time of *Anopheles stephensi*. Jeyabalan *et al*., (2003) using methanol extract of *Pelargonium citrosa* leaf against *An. stephensi*, Nathan *et al*. (2005) using the neem *Azadirachta indica* extract against *A. stephensi* and Nathan *et al*. (2006) using methanolic extracts of leaves and seeds from the chinaberry tree *Melia azedarach* against *A. stephensi*.

Also, Promsiri *et al.*, (2006) found that the first instar larvae were more susceptible to *Annona muricata* and the second instar larvae more susceptible to *Anethum graveolens* then the third and fourth instar larvae instar. Our results agree with those obtained by Okumu *et al*.,(2007) stated that the concentration of 6 ppm of *Azadirachta indica* oil inhibited the Adult emergence by 50%, significant reductions on growth

indices and pupation, besides prolonged larval periods, were observed at neem oil concentrations above 8 ppm. Hosana *et al*., (2007) discussed the potential value of extracts and amides derived from *Piper tuberculatum* as efficient insecticides against the larvae of *Anticarsia gemmatalis*, they found that extracts of seeds, leaves and stems of *P. tuberculatum* caused 80% mortality. Our results also agree with those obtained by with Jbilou *et al*.,(2008) who stated that the *Raphanus raphanistrum* and *Peganum harmala* extend the larval period of *Tribolium castaneum* when compared to the control. Murthy and Rani (2009) reported that the acetone extract of *Piper cubeba* and *Capparis spinosa* showed significant larval mortality against the yellow fever mosquito, *Aedes aegypti*.

Adetailed laboratory study on extracts of fruit of *Piper nigrum* against larvae of *Culex pipines*, *Aedes aegypti* and *Aedes togoi* was carried out Park, *et al*., (2002), Remia and Logaswamy (2010). The authors determined the LC_{50} and observed the behavioral changes and mortality in the larvae. Similar observations were noticed in the present study and support the potential applications of these herbs in insects control measures. Mansour *et al*., (2011) reported the larvicidal activity of *Piper nigrum* against the larval stage of *Musca domestica*, the larval, pupal duration recorded high significant decrease in the treatment larvae compared with the control, also reduced the pupal weight and the pupa failed to develop into adult stages. Kumar *et al*., (2011) reported that the neem seed kernel extract and Spinosad have displayed toxicity on different larval instars of *A. stephensi* and *C. circumdatus*. The study showed an increase in mortality with the increase in concentration and the early instar larvae are much susceptible than the later ones. Also, our results in agreement with Kalaivani *et al*., (2012) who reported the biological activity of selected Lamiaceae and Zingiberaceae plant essential oils against the dengue vector *Aedes aegypti* and found that the early instars were more susceptible than the late instars and pupae.

Kovendan *et al*.,(2012) reported that the *Carica papaya* leaf extract showed larvicidal and pupicidal effects after 24 h of exposure; however, the highest larval and pupal mortality was found in the leaf extract of methanol *C. papaya* against the first- to fourth-instar larvae and pupae of values LC_{50} = I instar was 51.76 ppm, II instar was 61.87 ppm, III instar was 74.07 ppm, and IV instar was 82.18 ppm, and pupae was 440.65 ppm, respectively. This is an ideal eco-friendly approach for the control of chikungunya vector, *A. aegypti* as target species of vector control programs. Also, Govindarajan *et al*., (2013) determined the Mosquito larvicidal activity of thymol from essential oil of *Coleus aromaticus* Benth. against *Culex tritaeniorhynchus*, *Aedes albopictus*, and *Anopheles subpictus* (Diptera: Culicidae) they found the larval mortality was observed after 24 h of treatment. The thymol had a significant toxic effect against early third-stage larvae of C. tritaeniorhynchus, A. albopictus, and A. subpictus with an LC50 values of 28.19, 24.83, and 22.06 µg/mL respectively,

Also, our results in agreement with Rajeswary and Govindarajan (2014) who determined the adulticidal activity of hexane, benzene, chloroform methanol leaf and seed extracts of *Delonix elata* against *Aedes aegypti*, the highest adulticidal activity was observed in the leaf methanol extract of *D. elata* against *A. aegypti* with the LC50 and LC90 values 162.87 and 309.32 ppm respectively. Sakthivadivela *et al*.,(2014) determined the larvicidal activity of crude aqueous and petroleum ether extracts of *Wrightia tinctoria* fruits and leaves against the filarial vector, *Culex quinquefasciatus*, the plant parts tested, aqueous fruit extract exhibited highest larvicidal activity followed by aqueous leaf extract.

Conclusion:

Limonene was the major compound in the essential oil of *P. nigrum* fresh fruit. The water extract of *Piper nigrum* fruits was more effective in controlling the flesh fly *Sarcophaga haemorrhoidalis*. In general, it could be concluded that almost the plant extract used in the present study act as larvicidal, and possess growth and emergence inhibiting factor against *S. haemorrhoidalis*. Furthermore, the results of the present study may contribute to a reduction in the application of synthetic insecticides, which in turn increases the opportunity for natural control of various medically important pests by botanical pesticides. Further studies on the tested plants including mode of action, synergism with the biocides under field condition are needed.

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