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Comparative Bioactivity of Peganum Harmala (Zygophyllaceae) Leaves, Flowers and Seeds Aqueous Extracts on Drosophila Melanogaster (Diptera: Drosophilidae): Mortality and Growth Inhibition Impact

COMPARATIVE BIOACTIVITY OF *PEGANUM HARMALA* (ZYGOPHYLLACEAE) LEAVES, FLOWERS AND SEEDS AQUEOUS EXTRACTS ON DROSOPHILA MELANOGASTER (DIPTERA: DROSOPHILIDAE): MORTALITY AND GROWTH INHIBITION IMPACT

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ABSTRACT

Many plant essential oil, extracts and phytochemicals are known to possess toxic activity against *Drosophila melanogaster*. In the current work, the effect of aqueous leaves, flowers and seeds extracts of *Peganum harmala* on the fruit fly mortality and larval development were determined. The seeds extract was the most effective; 100 % of mortality was recorded for the highest concentrations (200 and 300 µg/ml) with lethal time values (LT_{50} and LT_{50}) reached 3.72 to 4.47 and 6.61 to 8.51 days, respectively. In the other hand, the 15-day lethal concentrations, LC_{50} and LC_{90}, were determined to be 61.66 µg/ml and 100 µg/ml. Moreover, the use of the leaves extract significantly increased the mortality (71.1 %) and minimum mortality (12.5 %) was recorded with flowers extract. All extracts affected the final number of individuals per stage causing an elongation of metamorphosis. The growth-inhibiting effect is dose-dependent as expressed by significantly FNO that is always negative for the treated series. The T_{50} values of pupation and adult emergence were observed on the 3rd to 8th days and 12th to 11th days in application groups with leaves and flowers extracts, comparing to only 02 and 07 days in control. In addition, severe morphological malformations and anomalies in different organs of the three stages were recorded.

Keywords: *P. harmala, D. melanogaster*, mortality, growth inhibition, aqueous extract, bioactive compounds.

INTRODUCTION

Recently, researches have been widely focused on benefits and potentially toxicological properties of aromatic plants (Alexander et al., 2019). Plants contain an ample of bioactive chemical compounds known as secondary meTableolites (Ventrella et al., 2016). Generally, they are synthesized in small quantities and considering like a form of adaptation and defense strategy against herbivores (Bennett and Wallsgrove, 1994; Chowanski et al., 2016). Thus repel, inhibit growth or kill pests (Hikal et al., 2017). The classification of this variety of compounds is basing on several criteria; the chemical structure, the composition, the solubility in organic solvents or water, and their biosynthetic pathway (Osbourn, 1996; Crozier et al., 2006). Qualitative and quantitative reports of these components are species specific and could be an important measure in plant taxonomy **(**Croteau et al., 2000). A wide variety of biological properties was reported for genus *Peganum*. Among them is, its usage in pest management in countries of the Sahara regions especially *Peganum harmala* (Bitchagno et al., 2022). This species belongs to Zygophyllaceae family and it is commonly found in the Mediterranean region and Southeastern Europe (Bitchagno et al., 2022). In the

scientific literature, it was confirmed that the plant provides a great toxicity throw different extracts, yet it knows therapeutic properties; abortive, hypnotic, analgesic, antiseptics antitumor as well as antimicrobial and antifungal (Apostolico et al., 2016).

In researches, seeking plant extracts bioactivity; it is necessary using a model organism that displays how the compounds reveal this bioactivity (Silva and Wohlenberg, 2009). The fruit fly *Drosophila melanogaster* (Diptera: Drosophilidae) is considered one of the best models, because is widely used to research problems requiring multidisciplinary approach (Rubin, 1988). *D. melanogaster* is easy to handle, has a short life span, requires small space and allows large number of repetitions, which assure good statistical precision (Graf et al., 1984).

The main object of this work was to compare the efficiency of aqueous extracts from different parts of *P. harmala* (leaves, flowers and seeds) and their potential toxicity in *D. melanogaster*. Secondly, we evaluated the possible adverse effects on life span and developmental disorders of the insect.

MATERIAL AND METHODS

Drosophila Stock and Culture

The initial *D. melanogaster* stock population (Wild type) was picked up from fermented apples in region of Annaba (Algeria). The flies were fed continuously on a SDM (Standard *Drosophila* Medium) containing of cornmeal-yeast-agar, maintained under controlled conditions (T: 25 ± 1°C, H: 70 % and a 12:12h L/D cycle).

Plant Collection and Preparation of Simple Extracts

P. harmala (Zygophyllaceae) leaves, flowers and seeds were collected from, Laghwat town in Algeria, a Saharan and Arid region where it grows mainly. It is located 329 km south of Algiers and 750 m above sea level (33°48′10″N, 2°52′30″E).

To make cold aqueous extracts, 500 g of each part were taken and thoroughly washed in distilled water. Separately, they were deposited in 1L of distilled water for boiling on hot plate at 180 °C (1 h of time), until obtaining initial stock solutions of 1 g/l. Final suspensions were sterilized by filtration with membrane filter (Whatman paper). Filtrates were stored at refrigerator until used.

Mortality Bioassay

Mortality bioassay was conducted using the three plant extracts (leaves, flowers and seeds) with three to four different concentrations for each. Every concentration (10 ml) was mixed into 40 g of SDM. For control, we used vials with only SDM. In each trial, 20 at 30 larvae of the same age (second instar) of *Drosophila* were introduced from synchronized cultures. Larval mortality was observed quotidianly for 15 days. The trials were repeated at four times for both control and treated series (per concentration).

Development Assays

For 15 days, we counted the number of survival individuals per stage (third instar larvae, pupae and imagoes). The various malformations obtained are also recorded and photographed using Stero Lumar V12.

The T_{50} or the 50 % of developmental time was calculated for pupal and imaginal stage, which signifies time (in days) required to 50 % of the tested larvae population to reach the next stage of development. It demonstrates potential variation in the developmental time after exposure to tested extracts.

According to Ventrella et al., 2016, the FNO factor or the final number of organisms was determined comparing to

control. It is defined by the following formula:

$$FNO = \frac{T - C}{C} \times 100$$

Where, C: indicates number of survival organisms counted in control medium, and T: indicates number of survival organisms counted in treated medium.

The final number of individuals with high values in the treated series that the control corresponds to a positive FNO and, in case of a lower number, in the treated series than the control, corresponds to a negative NAO.

Data Analysis

Mortality values were corrected by Abott's formula from the observed data. Then, they were normalized and processed using Bliss Tableles. According to Finney's mathematical methods, lethal concentrations and lethal times (LC_{50}, LC_{90}, LT_{50} and LT_{90}) were determinated (Finney, 1971).

In order to compare the mortality rate and development parameters, the ANOVA test (One way analysis of variance) was utilized on XLStat 2014 Software. Graphs showing survival rate and individual numbers were also drawn using the Microsoft Windows Office-Excel program.

RESULTS

Mortality and Toxicity Bioassay

Initially, we studied the toxicological impact of *P. harmala* aqueous extracts from different parts. They show a good insecticidal activity and cause a significant mortality depending on concentration and time of exposure. After 2 days, the larvae are not very sensitive to *P. harmala* where mortality rates do not exceed 16 % (Table 1). However, they succumbed to treatment as early as day five. Contrarily the floral extract, those of leaves and seeds showed more mortality with a rates varying between 16.66 % to 42.22 % and 66.3 % to 93.8 %, respectively, in 10 days of treatment. Furthermore, the highest percent-corrected mortality was found in seeds extract (100 % at 200 and 300 µg/ml), at the end of the test. Highly significant differences were revealed by the analysis of variances (F_{obs}= 12.88; $p < 0.002$) (Table 2), at significance level $\alpha = 0.05$, followed by the leaves extracts that exceeded in causing more than 71.1 % and least by flowers extract (12.5 %) at the highest concentration as shown in (Table 2).

In the respect that seeds extract showed the highest efficiency on *D. melanogaster* larvae, its toxicological parameters were represented in Tablele 4. Data reveals that the mortality rate is strongly correlated with the exposure time of flies to different concentrations after 02 to 10 days of treatment. The 15-day lethal concentrations, LC_{50} and LC_{90} were determined to be 61.66 µg/ml and 100 µg/ml respectively. Linear regression for percent-corrected mortality clearly revealed that extract tested exhibited concentration dependent activity against flies. As shown in Tablele 04, lethal time (LT_{50}) that can kill 50 % of larvae reached 3.72 to 4.47 days for the highest concentrations (200-300 µg/ml). To combat 90 % of population, it takes 6.61 and 8.51 days respectively (Table 4).

The previous test is followed by analysing the deffered effects of the three plant extracts on *D. melanogaster* development cycle. A repeat control group was used for each extract groups. Developmental time was followed from second instar larvae stage to adult eclosed. All extracts affected the final number of individuals and caused an elongation of metamorphosis (Table 5). Comparing to control, survivals number were decreased until 8.66 and 17.00 using leaves and flowers extracts with higher concentrations.

Table 1: Effect of *P. harmala* leaves aqueous extract on mortality rate of *D. melanogaster*

Days	2	5	10	15	F_{obs}	*P*
50µg/ml	3.3 %	3.3 %	3.3 %	3.3 %	**0**	**1**
300µg/ml	1.11 %	3.33 %	16.66 %	27.8 %	**0.69**	**0.58**
1000µg/ml	15.55 %	22.22 %	42.22 %	71.1 %	**1.15**	**0.38**
F_{obs}	**0.26**	**1.91**	**1.13**	**1.26**		
P	**0.78**	**0.23**	**0.38**	**0.35**		

[*: Significant, **: Highly significant, ***: Very highly significant]

Table 2: Effect of *P. harmala* flowers aqueous extract on mortality rate of *D. melanogaster*

Days	2	5	10	15	F_{obs}	*P*
250µg/ml	0 %	2.50 %	5 %	5 %	**3.43**	**0.05***
500µg/ml	2.5 %	3.75 %	3.75 %	5 %	**0.60**	**0.63**
750µg/ml	6.25 %	5 %	5 %	8.75 %	**1.43**	**0.28**
840µg/ml	7.5 %	7.5 %	7.5 %	12.5 %	**4.19**	**0.0001****
F_{obs}	**5.81**	**2.90**	**1.51**	**1**		
P	**0.01***	**0.08**	**0.26**	**0.43**		

[*: Significant, **: Highly significant, ***: Very highly significant]

Table 3: Effect of *P. harmala* seeds aqueous extract on mortality rate of *D. melanogaster*

Days	2	5	10	15	F_{obs}	*P*
50µg/ml	0 %	2.5 %	16.3 %	26.3 %	**4.96**	**0.02***
200µg/ml	15.05 %	63.8 %	93.8 %	100 %	**44.45**	**0.0001****
300µg/ml	15.07 %	31.3 %	66.3 %	100 %	**2.70**	**0.09**
F_{obs}	**3.52**	**6.32**	**2.20**	**12.88**		
P	**0.08**	**0.02***	**0.17**	**0.002****		

[*: Significant, **: Highly significant, ***: Very highly significant]

Table 4: Toxicological parameters related to aqueous extract of *P. harmala* seeds on *D. melanogaster*

	Regression line		**LC_{50} (µg/ml)**	**LC_{90} (µg/ml)**
2 days	Y= -9.10 + 5.45X	R^2= 0.96	386.50	636.77
5 days	Y= -0.85 + 2.39 X	R^2= 0.70	281.84	954.99
10 days	Y= 0.12 + 2.42 X	R^2= 0.62	104.71	354.81
15 days	Y= -5.71 + 6X	R^2= 0.95	61.66	100
	Regression line		**LT_{50} (days)**	**LT_{90} (days)**
50µg/ml	Y= -1.12 + 5 X	R^2= 0.93	16.60	30.20
200µg/ml	Y= 2.14 + 5.04 X	R^2= 0.92	3.72	6.61
300µg/ml	Y= 1.93 + 4.69 X	R^2= 0.71	4.47	8.51

[*LC: Lethal concentration, LT: Lethal time, Y: Probit mortality, X: Concentration/Time decimal logarithm*]

Results revealed that seeds extract was the most powerful where no organism survived at 200 and 300 µg/ml with FNO equal to -100. Their effect is concentration-dependent while FNO parameter appeared significantly negative for the treated groups (Table 5). In addition, developmental stages were delayed for all concentrations of *P. harmala* (larvae-to-adult). Particularly, for 1000 and 840 µg/ml in application groups with leaves and flowers extracts, 50 % of pupae were observed on the 3rd and 8th days, respectively. After 2 days of treatment, pupation was recorded in the control groups. Fifty per cent of adults were formed on the 12th and 11th days, respectively, at the same concentration comparing to only 07 days in control (Table 5). In the other hand, seeds extract deleted definitively both stages at 200 and 300 µg/ml.

Table 5: Effect of larval exposure to *P. harmala* on development of *D. melanogaster (M ± SEM)*

		T_{50} Pupae	T_{50} Adults	N° final organisms	FNO
	Control	2.00 ± 0.00	7.00 ± 0.00	30.00 ± 0.00	0.00
Leaves [1]	**50µg/ml**	3.00 ± 0.00	9.00 ± 0.00	29.00 ± 0.67	-3.33
	300µg/ml	2.33 ± 0.44	8.67 ± 0.89	21.67 ± 3.78	-27.77
	1000µg/ml	3.33 ± 0.44	12.33 ± 1.56	8.66 ± 3.36	-71.13
	Control	2.00 ± 0.00	7.00 ± 0.00	20.00 ± 0.00	0.00
Flowers [2]	**250µg/ml**	4.25 ± 0.38	9.75 ± 0.38	19.00 ± 0.50	-5.00
	500µg/ml	4.25 ± 0.38	10.75 ± 0.38	19.00 ± 0.50	-5.00
	750µg/ml	5.25 ± 0.75	10.50 ± 1.00	17.00 ± 1.50	-1.50
	840µg/ml	8.00 ± 0.50	11.25 ± 0.75	17.50 ± 1.00	-12.50
	Control	2.00 ± 0.00	7.00 ± 0.00	20.00 ± 0.00	0.00
Seeds [3]	**50µg/ml**	2.25 ± 0.38	5.25 ± 0.38	14.75 ± 0.75	-26.25
	200µg/ml	/	/	0.00 ± 0.00	-100
	300µg/ml	/	/	0.00 ± 0.00	-100

[*[1]: (N=30; R=03), [2,3]: (N=20; R=04), M: Mean, SEM: Standard error of the mean*]

The ranking of recovery effect of the three extracts of *P. harmala* was: Seeds > Leaves > Flowers. The next Figureure represent the detailing repartition of larvae, pupae and adults stages of the survivals under effect of seeds aqueous extract at 02, 05, 10 and 15 days of treatment. According to above results, significant differences were recorded between the treatment and control groups ($P < 0.05$) (Figure 1).

In our experiments, we recorded severe morphological malformations and anomalies in different organs of the three stages followed by death such as burned larvae, dead adults inside pupae (Figure 2). Especially, those observed in adults: shortened legs due to fusion of tarsal segments, unopened wings, lack of thorax formation, smaller body size or smaller abdominal black zone, and changes in body pigmentation (Figure 2). The control organisms do not have the same malformations previously described.

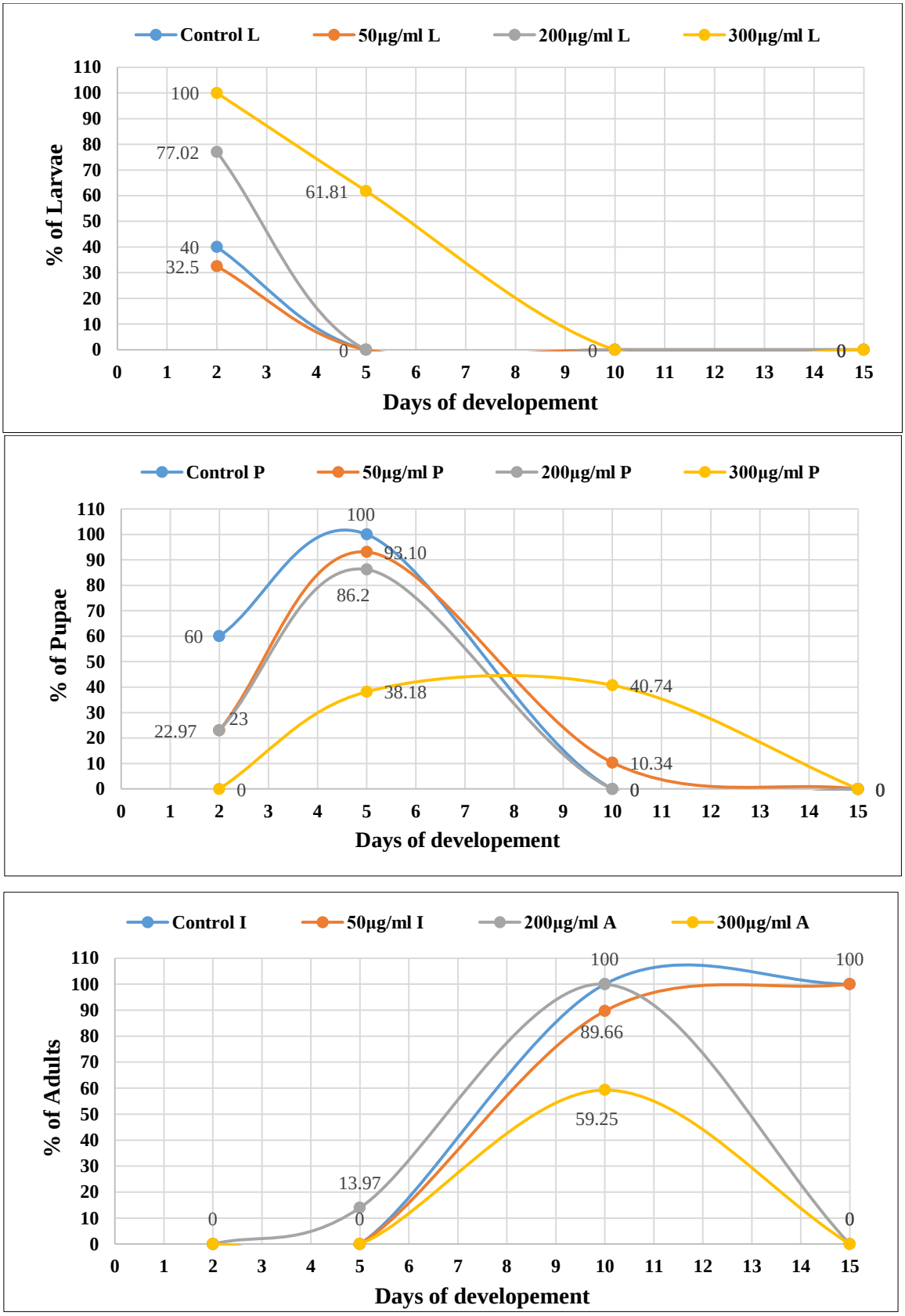

Figure 1: Comparison of *D. melanogaster* developmental stages under effect of seed's aqueous extract.

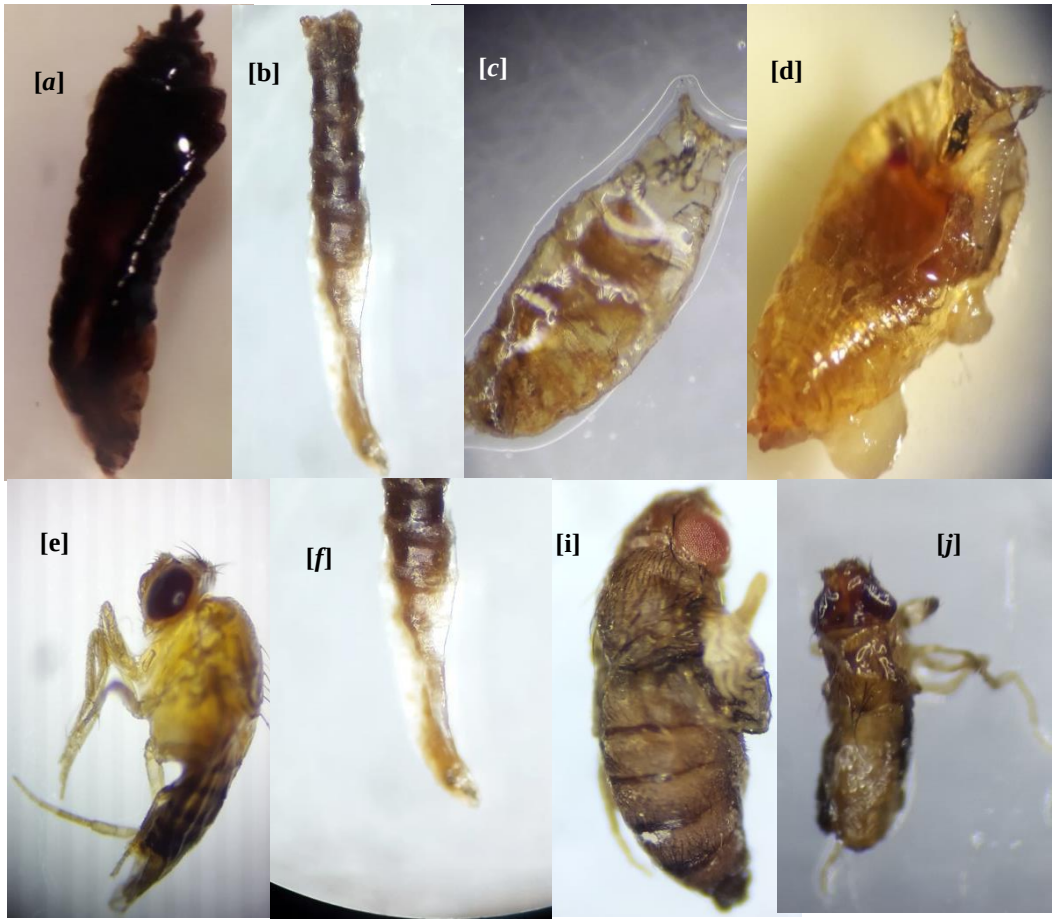

Figure 2: Examples of the most frequent malformations in *D. melanogaster* caused by *P. harmala* aqueous extracts (x 4, 5).
[*a, b :* *burned larvae* *; c, d :* *dead adults inside pupae* *; e :* *smaller black abdominal zone* *; f, i :* *deformed wings* *; j :* *smaller body size shape & shortened legs*].

DISCUSSION

Besides to the various pharmacological applications of plant extracts with different modes of action because of their richness in phytochemicals compounds, they are also considered a good source of high toxicity (Araújo Pinho et al., 2014). Thus, nowadays biopesticides of vegetal origin has a primordial place in pest management interventions including insects (Anjum et al., 2010 ; Alves et al, 2014 ; Riaz et al., 2018).

The current investigation reports the potential toxicity of *P. harmala* using aqueous extracts from different parts against *D. melanogaster*, as well as a guideline model. Our results demonstrated that it manifested a great insecticidal power with a significant concentration-response relationship. Unlike the floral extract, those of leaves and seeds have a significant toxicity with a mortality rate varying between 71.1 and 100 %. At the highest concentrations of seeds extract (200-300µg/ml), which had major effect among all the treatments, LT_{50} that can kill 50 % of larvae reached 3.72 to 4.47 days. To combat 90 % of population, it takes 6.61 and 8.51 days respectively. 15-day of exposure, LC_{50} and LC_{90} were determined to be 61.66 and 100 µg/ml.

Similarly, the efficiency of different extracts, natural and synthetic compounds of *P. harmala* is described to occur toxicity to a large range of pests (Bitchagno et al., 2022). We mention as example : the flower bugs *Frankliniella occidentalis* (Razavi and Ahmadi, 2016), the termites *Heterotermes indicola* (Aihetasham et al., 2015), the beetles

Trogoderma granarium (Zeinab and Abdelhafiz, 2019) and *Tribolium castaneum* (Ahmed et al., 2022), the diamondback moth *Plutella xylostella* (Abbasipour et al., 2010), the crickets *Locusta migratoria cinerascens* (Benzara et al., 2012), the mosquitos *Aedes aegypti* (Yang et al., 2020) and *Culex pipiens* (Benhissen, 2016), …etc.

For the control *Sitophilus oryzae* L the rice weevil, Dhumad et al. (2015) applied different *P. harmala* seed extracts at a concentration of 1 %, 2 % and 3 %. After 24, 48, 72 hours, mortality rates of 98, 100, 100 % were recorded with the ethanolic extract and 64, 66, 74 % with an aqueous extract, respectively (Dhumad et al., 2015). The efficacy of *P. harmala* (10 min decoction) was tested toward different developmental stages of *Bemisia Tableaci*; the immature pests were more sensitive, with 50 % mortalities, than the adult stage (Al-mazra'awi and Ateyyat, 2009).

Comparably with the research of Ismahane et al. (2016), where the topical application of essentials oils on forth instar larvae of the date moth *Ectomyelois ceratoniae* attained 56.66 % of mortality (5 days of treatment), with a LT_{50} of 2.57 days. For the same period of exposure, a rate of 100 % mortality was obtained with a LT_{50} of 1.45 days in adults (Ismahane et al. 2016).

Our study has also demonstrated that *P. hamala* interfere directly through all phases of life cycle in *D. melanogaster* and interrupting insect's normal physiology, when they were fed on diet in which it was incorporated. The aqueous extracts affect significantly the emergence of larvae, pupae and adults causing an elongation of each stage or inhibiting molting. Naturally, with increasing the concentrations, the growth-inhibiting effects were increased. A delay of larval molting 3 to 8 days (T_{50} of pupation) as well as adult emergence 8 to 12 days has been registered. The seeds extract was proved most effective as it showed the FNO parameter (-100 %) where the final number of organisms was totally suppressed and individuals could not reach the next phase. The larvae, which survived the treatment, underwent both morphological and physiological disturbances that lead to several deformations of the various organs, significant reduction in size, and a change in pigmentation.

It has been reported that the use of different plants including *P. harmala* reduced growth of the *T. castaneum* (Jbilou and Sayah, 2008 ; Sagheer et al., 2014). Similarly, acetone extract of *P. harmala*, at 15 % concentration, caused 75.15 % of population inhibition (Kanwal et al., 2021). Regarding growth inhibition, results demonstrate that lowest numbers of larvae (57.33), pupae (39.00) and adults (32.00) were emerged comparing to maximum number of organisms (110.00) emerged in control test (Kanwal et al., 2021). Rehman et al. (2009) have shown that 0.2 % *P. harmala* ethanol extract of seeds incorporated with feeding to *Bactrocera oleae* adults had several negative effects. It provoked egg deformations blocking their development, reduced the number of surviving progeny and extended offspring growth (Rehman et al., 2009).

The ability to use *P. harmala* as a botanical insecticide comes from its richness and diversity of chemical constituents such as; alkaloids, flavonoids, anthraquinones, triterpenoids, phenylpropanoids, amino acids, carbohydrates and some volatile constituents (Kartal et al. 2003 ; Li et al., 2017). In the leaves, the existence of tannins, steroids and saponins has been demonstrated through a phytochemical screening (Pahlavia et al., 2018). A catecholic B ring of flavonoids is a major responsible for the toxicological activity in *P. harmala* (Onyilagha et al. 2004). Flavonoids are mostly abundant in the aerial parts of the plant (Sharaf et al., 1997). The quinazolines and β-carbolines are the main types of alkaloids (Li et al.,

2017). The highest levels of them is dispersed in ripe seeds and roots, followed by leaves and stems with low levels and non-existent in flowers (Kartal et al., 2003 ; Abbasipour et al., 2010 ; Asgarpanah and Ramezanloo, 2012), which prove our findings.

It was confirmed that different extracts of *P. harmala* and its isolated alkaloids derivatives induce the inhibition of acetylcholinesterase enzyme (Zheng et al., 2011; Yang et al., 2015). A recent study of Miao et al. (2020) has underlined the mechanism of *P. harmala* seeds on *C. elegans* central nervous system. A decrease of AChE activity has been recorded under effect of the main alkaloids of the plant, namely, harmol and harmaline; 30.61 U/mg pro in control, 12.36 U/mg pro in case of exposure to 1.00 mg/mL of ethanol extract (Miao et al., 2020). This was associated with sublethal effects as an interruption of locomotion behavior, decrease of lifespan and body length (Miao et al., 2020).

P. harmala extracts also induce the inhibition of enzymatic activities and this has been demonstrated in several cytological studies. According to Bouayad et al. (2013), methanol extracts of *P. harmala* has been evaluated on physiological parameters of *Plodia interpunctella* larvae: detoxification enzymes, hydrolytic enzymes in midgut activities and reserve substances. Results reveal a significant reduction in larval contents of carbon hydrate and protein (Bouayad et al., 2013). In the same way, Jbilou et al. (2008) indicated that seeds extracts reduce amounts of different substances (glycogen, protein, and/or lipid) in *T. castaneum* larvae.

Previous study on *S. litura* has already shown that *P. harmala* causes a decrease in the body sugar content and the protein of the hemolymph of the insect (Sun et al., 2004). In addition, Bouayad et al. (2013) found that *P. harmala* exhibited inhibitory activity against enzymes, α-amylase and proteases, in *P. interpunctella* larvae. Moreover, it induced the activity of detoxification enzymes, glutathione S-transferases and esterases, cytochrome P450 monooxygenases and esterases (Bouayad et al., 2013). Indeed, it occasioned a serious damage in cells of midgut epithelial, occurring a cytoplasmic vacuolization and extend the intercellular space (Jbilou and Sayah, 2008).

CONCLUSION

From these results, it was concluded that different parts of *P. harmala*, especially seeds, are effective to process a strong insecticidal activity against *D. melanogaster* larvae. Because of its various bioactive compounds, this plant could be useful as growth inhibitor for suppressing insects populations. Thus, it has an excellent potential to be utilized as a naturally occurring agent for pest management strategies.

AUTHORS CONTRIBUTION

All the authors contributed equally in this research trial.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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