

# Evaluation Of The Efficacy Of Some Plants Extract Against The House Fly, Musca Domestica (Diptera : Muscidae) And A Number Of Insect Pathogens

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#### Abstract

The results of the study showed that the phenolic extract of the plants used effected the various life stages of the house fly Musca domestica , the percentage of eggs mortality were (76,70, and 37.33)% when using the highest concentration (500)ppm for the extract of Eucalyptusobliqua, , Haloxylon salicornicum and Mantha piperita respectively, as for the sensitive of the larval stages, as the mortality rate was (93.33, 86.66 and 74.44)% when using the highest concentration of Eucalyptus, and respectively, percentage pupae mortality were (40, 33.33 and 24.44)%, as for its effect on adults, the mortality rate it used the same higher concentration was()% for the mentioned extracts, respectively. As for the attractive and repellent effect all extracts showed a repellent effect, all extract did not record any effect on types the bacteria (Bacillus subtilius, B. sphaericus, B. lentimorbus and B. poppiliae), While they were causing inhibition on of the growth of isolated entomopathogenic fungi.

Keywords: Musca domestica , Eucalyptus oblique , Haloxylon salicornicum , Mantha piperita.

#### 1. Introduction

Chemical pesticides such as chlorine and phosphorus pesticides are one of the pillars of environmental pollution due to the persistence of some of these pesticides in soil and water and the negative impact of pesticides in human and animal health, as well as reducing the density of natural enemies, which plays a large role in the balance of the ecosystem, Because the domestic fly insects is medically important because of its spread and its link to humans, it transmits many pathogens in mechanical ways, as it has been shown to be responsible for the transmission of more than 100 pathogenic causes such

as the parasite of the Amoeba Entamobae histolytica, Salmonela typhi Cholera, as well as transmission of parasitic worms to humans [1]. Which led researchers to depend on the chemical control of this insect and the use of alternative methods, including plant extracts, which are characterized by rapid decomposition and instability in the environment for a long time and lack of toxicity to humans and beneficial organisms as well as the lack of insects to acquire the resistance against them and important plants Eucalyptusobliqua, which it considers rich source of active compounds. The plant contains preservatives and contains pyrocatechine, catechine, kinoin and kinored, these are all phenolic substances [2], Haloxylon salicornicum and Menthapiperita, where these plants contain toxic substances, volatile oils, resinous substances, repellents and phenolic materials [3], Nagueira [4] had found that the extract of the seedling plant was highly effective in the expulsion of M. domestica, The aim of the study was to evaluate the toxicity that effects of phenolic extracts of E.oblique , H.salicornicum and M. piperita Different life stage of M.domestica, entomopathogenic bacteria: B. subtilius, in B.sphaericus, B. lentimorbus, B. poppiliae, and a number of pathogenic fungi and used as effective plant fertilizers and available as natural resources and less polluting the environment.

# 2. Materials and Methods

#### 1- Culture of Insect

Adult insects were raised in glass cages and removed  $(40 \times 37 \times 37 \text{ cm})$  in diameter with a circle hole allowing the entry of the hand to deal with insects. Adults were feeding to a medium containing water and milk , placed in a petri dish with a piece of cotton. The eggs of the insect were collected by small forceps and then placed in a petri dish containing another medium for larval culture consisting of 600 g of cow dung, 200 g of meat extract, 20 g of yeast powder and 1200 ml of distilled water [5]. The petri dishes were placed in the laboratory until they were using in experiments . The resulting larvae were completed in special petri dishes until they were used in subsequent experiments.

# 2- Collection and diagnosis of plant samples

Plant specimen were collected from the gardens of Al- Diwaniyah city and were identified by Assistant Professor Dr. Suhaila Hussein, Faculty of Education, University of Qadisiya.

# 3- Preparation of plant extracts

The parts of plants which they used were sprayed and the phenolic extracts of each plants , were scavenged according to the method of [6]. Take 10 g of dry vegetable powder and put it in a 100 mL glass flask and add 40 ml from acetic acid 2%. The extraction was carried out by the inverter condenser using an 80 m water bath for 8 hours. Then it should be to cool down and then sprayed with a filter paper by adding a suitable volume of propanol and saturated the solution by adding a quantity of sodium chloride and a well-formed two layers. The upper layer was containing the phenol compounds was isolated using the separation funnel and dried with the rotary and , by taking with 0.5 gm of the powder extract and solvent in 50 ml from acetic acid and complete it to 1000 ml by adding distilled water , the concentration were prepared ( 100, 300, 500) ppm .

## 4- Study the effect of phenolic extracts on egg hatching

Use 1 to 12-hour eggs with 30 eggs and 3 replicates for each concentration. Eggs of 5 mL of each concentration (100, 300, 500) ppm of phenolic extract by a 10 mL hand spray. Transfer the treated eggs to 100 ml plastic containers containing pre-prepared food medium for larval growth and breeding. The pots were covered with a cloth of tulle to ensure that the larvae did not leave the pots after had hatched. The pots were incubated at 28 ° C. The mortality rate was recorded after 48 hours of treatment and the values of the loss were corrected according to [7]. As for the control treatment, the eggs were sprayed with only 5 ml of distilled water .

corrected mortality % = percentage of mortality in treatment- % percentage of mortality in control

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100 --percentage of mortality in control

# 5- Study the effect of phenolic extracts in the larvae phases

A study of the effect of phenolic extracts in the larval instars was adopted by a modified method of the study Morsy [8] where the larval instars food was treated with phenolic extracts for each plant separately and according to the concentrations used by replicates for each concentration and then put 10 larvae of each stage to be grown separately in a 9 cm diameter Petri dish containing the 2 mL of phenolic extract with 1 ml of 80 Tween as a diffuser and put in the incubator at 28 ° C. The treatment of control was replaced by added extract to the nutritional medium with distilled water recorded losses after 48 hours of treatment and corrected the values of the percentage mortality

# 6- Study the effect of phenolic extracts in the pupae stage

Fifteen pupae from larvae that treated with phenolic extract were taken by three replicates for each concentration in addition to control and placed in a petri dish incubated in the same of conditions which they referred to previously. The percentage of mortality was recorded and the values of the mortality were corrected.

### 7- Study the effect of extracts Phenolic in the adults

Numbers of adults were obtained from the development of the first phase larvae treated with phenolic extract concentrations and the adult ages of males and females were calculated.

#### 8- Studying the attractive and chasing effect of adults.

As for the attractive and chasing effect of the mentioned extracts on adult insects was using the device of chemical affiliation [9]. It consists of a wooden box  $(48 \times 20 \times 20 \text{ cm})$  in which two opposite openings of the sides pass through a 100 cm open pipe. In the center of the pipe there is an opening to insert the insects into the right end of the pipe with a piece of cotton treated with the concentration of phenolic extract for each plant separately. With a wet cotton with distilled water only. When returned as a control agent and put ten eggs in the middle of the tube. waited for 20 minutes to calculate the number of insects drawn from the material. The experiment was treated according to a normal laboratory conditions. The tube was cleaned between the treatment and the other by removing the treated cotton and cleaning the tube. Each treatment was repeated with three replicates. The. results were then calculated according to the following equations [10].

Percentage of attraction = The number of insects traveling in front of the tested material and cut 25 cm at the center× 100

Total number of insects

Percentage of expulsion = The number of insects inverted against the tested material and cut 25 cm from the center  $\times$  100

Total number of insects

9- Vital assessment on bacteria

In the test, use the method of Disk diffusion on filter paper according to method [11], to test the effectiveness of phenolic extracts with a concentration of 100 ppm on the bacteria under studying, the test was as follows :

-Concentrate and prepare a concentration of 100 ppm of all phenolic extracts

#### • Source of bacteria

The four types of bacteria (B. subtillus, B. sphaericus, B. lentimorbus and B. poppiliae) were isolated from the soil, 40 samples were collected from agricultural, nonagricultural soils and gardens. The samples that were able to grow on the sodium selective were diagnosed and dyed with coomassie brilliant blue stain.

The growth environment of the bacteria is a type of Nutrient agar 39.5 g/1 liter in petridish 20  $\mu l$  .

#### • Preparation of bacterial suspension

Distilled water has taken to a small amount on the surface of the bacterial growth, and it was scoured with a tubing of the node, then poured into a liquid environment (Nutrient broth )and incubated at 37 ° C for 24 hours to ensure bacterial growth density as a center suspension. Then, take the 1  $\mu$ l suspension and pour in 9  $\mu$ l distilled water concentration 1  $\times 10^{-1}$  and from the latter 1  $\mu$ l is taken in 9 ml distilled water. Concentration of 1  $\times 10^{-2}$  and so on to reach the concentration of 1  $\times 10^{-4}$  which is the concentration required for isolation of the studied bacteria.

#### • Treatment

Sterile filtration papers (5 mm) were used and 40 microliters of eeach plant extracts concentration of 100 ppm were obtained and three treatments were applied in the dish. The fourth comparison dish was represented by three replicates for each incubator at 37 °C for 24 hours. The diameter of the inhibition area around the plant extracts was measured.

# 10- Vital assessment on fungi

The poisoned food method was used according to a method (Reyes et al., 1996) to test the effectiveness of phenolic extracts on the tested fungi with a concentration of 100 ppm as follows:

# • Source of fungi

For the purpose of isolating the fungi , 50 adults of M.domestica were taken , placed in sterile test tubes , and transferred to the laboratory . Sterilized with 5% sodium

hypochlorite solution for two minutes , then washed with distilled water then transferred by sterile forceps to plastic dishes with a diameter of 9 mm, containing the culture medium, Potato Dextrose Agar (P.D.A.) . The dishes were incubated at a temperature heat 25 °C for 7 days and the fungi were purified by taken 0.5 cm fungal growth from edge of the growing colony around the adult and transferred by a sterile cork piercing to the center of a dish containing P.D.A. fungi were identified using the taxonomic key [12].

# • Preparation of fungi

Modern cultivars of the fungus were prepared and 3ml corks are obtained from the growth of the fungi disk.

#### • Treatment

Add 0.2 ml from the main concentration of 100 ppm from each plant extracts in plastic dish and add 19.8  $\mu l$  from the cutler media P.D.A. This is equivalent to 3 ml. Then place the fungi tablet, which is newly grown in the middle of the Petri dish , and incubate for a different period according to the fungi type , three times for each extract with the comparison [13] , the percentage of inhibition is calculated by measuring the diameter in millimeters by equation .

100 % Inhibition = Hyphae growth in control - Hyphae growth in treatment × 100

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Hyphae growth in control

# 2.1 Stastical Analysis

The data were analyzed according to the design of the global experiments (C.R.D) and corrected the percentage of depreciation according to equation [14]. The least significant difference (L.S.D) was used in determining the statistical differences between the coefficients.

# 3. Results and Discussion

# Effect of phenolic extracts in eggs

Table (1) shows the rates of loss of domestic fly eggs after exposure to different concentrations of phenolic substitutes for E. obliqua, H. salicornicum and M. piperita, the high percentage mortality of egg were 76%, 70%, And 37.77% when used high concentration 500 ppm for the extract E. obliqua, N. oleander and M. piperita The results of the statistical analysis showed a positive relationship between concentrations

and percentage mortality. The cause of egg loss is due to the physical effect of these extracts in eggs, preventing gas exchange with the outer medium and hardening of the eggshell leading to the death of the fetus. This may be due to the effect of the extract on the embryos inside the egg and led to weak larvae that die when they released from the egg [15]. According to the results Kristensen [16], the phenolic extract of Eucalyptus camaldensis 5  $\mu$ g /ml. plant resulted in the loss of domestic fly eggs at a rate of 51-78.8 %. The phenol extract of the plant Nicotiana tabacum caused a loss 44.67% of Egg of white fly Bemesia tabaci.

Concentration		Mortality %	
ppm	E. obliqua	H. salicornicum	M. piperita
500	76.00Aa	70.00Ab	37.33Ac
300	63.33Ba	54.44Bb	23.33Bc
100	36.66Ca	30.00Cb	19.44Cc
Control	0.00Da	0.00Da	0.00Da

#### Table 1. Effect phenolic extract on egg of M. domestica

#### L.D.S(0.05) = 1.883

Different capital letters denote to significant differences vertically ( among concentrations), Different small letters denote to significant difference horizontally. Effect of phenolic extracts in the larval phases**2** -

Table (2) shows the effect of the phenolic extract of E. obliqua, H.salicornicum and M. piperita in the larval instar of the domestic fly, It was found that the first phase recorded the highest percentage mortality and significant difference from the second and third stages. The E.oblique extract achieved the best mortality of larvae at concentration of 500 ppm was 93.33% , followed by The phenolic extract of H. salicornicum and M. piperita plant showed the lowest effect of 86.66% and 74.44% at the same concentration of the first larval stage , The statistical analysis showed significant differences between the concentrations which they used and the three larval stages, with the first stage recording at 500 ppm, the highest percentage mortality, as a result of treatment with the phenolic extract of the E. oblique, while the third phase recorded the lowest concentration loss in 100 ppm , The result of the treatment with phenolic extract of the studied plant have achieved good results in the destruction of the larval instar. This activity is attributed to the number and efficiency of the phenolic compounds found in the chemical composition of these plants in the influence

of the larvae [17] confirmed the presence of effective phenolic compounds in the plant of Eucalyptus. The effect of plant extracts in insects is concentrated in the digestive system mainly because they are in the food medium of insects, [18] stressed that the chemical compounds extracted from the plants accumulate in the digestive tract and thus lead to the rupture of the lining membrane and the entry of these materials Toxic to the blood and spread in the body of the insect and the effect on the Important physiological processes , Sukantas et al, [19] pointed out that The treatment of larval food with plant extracts leads to the accumulation of chemical compounds found in them, such as phenols and their association with proteins and fats, which are difficult to digest complexes, which hinder the hormonal system and inhibits physiological processes and leads to the death of larvae, especially if these substances are highly toxic. Also, some of effective chemicals may reach nerve nodes in the body of the larvae and pupae affect the nerve impulses, which leads to inhibition of nutrition and imbalance of the body.

Matasyon et al, [20] mentioned that phenols combine with the digestive enzymes in the body of an insect such as protase, thus causing a decrease or discontinuation of the digestion process, resulting in reduced metabolism through the formation of hydrogen bonds between the groups of hydroxyl and nitrogen aggregates causing the breakdown of the basic enzymes in the larval body and thus death ,The results of this study are consistent with Nasser and Ali [21], who pointed out that the phenolic extract of the eucalyptus plant caused high mortality rates for domestic fly larvae. Kazem [22] added that the extract of the Nerium sp. plant caused 100% loss of larvae of the first stage of M. domestica The Biber nigerium extract resulted in the larvae killing 100% at 100 ppm [23].

	Mortality %								
Concentration ppm	E. obliqua			H. salicornicum			M. piperita		
	St1	St2	St3	St1	St2	St3	St1	St2	St3
500	93.33Aa	84.4Ab	73.33Ac	86.66Ab	76.66Ad	70.00Ae	74.44Ac	63.33Af	53.33Ag
300	80.00Ba	70.00Bb	63.33Bc	73.33Bd	70.00Bb	63.33Bc	60.00Be	53.33Bf	40.00Cc
100	63.33Ca	50.00Cb	40.00Cc	50.00Cb	40.00Cc	30.00Cd	40.00Cc	30.00Cd	26.66Ce
control	0.00Da	0.00Da	0.00Da	0.00Da	0.00Da	0.00Da	0.00Da	0.00Da	0.00Da

#### Table 2. Effect of phenolic extracts in larval stages of M .domestica

# L.D.S (0.05) = 2.861

Different capital letters dente to significant differences vertically ( among concentrations), Different small letters denote to significant difference horizontally. Effect of phenolic extracts on puppe **3** -

The results of the studies showed that the phenolic compounds have a good effect in the M. domestica pupae resulting from larvae treated with these extracts. Table (3) There were many abnormal cases in the form of pupa, as there were pupae of spherical, spherical or oval shape, as well as that a number of these pupae to adult and also recorded the appearance of adults incomplete growth of these pupae or with short wings and The results showed that the phenolic extract of E. obliqua has achieved many cases of abnormal pupa and it has been shown that many larvae that have not died as a result of the treatment of their food with extracts have been transformed into dead distorted pupae This means that the extracts have a cumulative effect in the insect body during their life cycle, The results of this studies are consistent with what is indicated . The phenolic extract of the melon plant led to the production of virulently distorted pupae in previous studies Nassir and Ali [24]. The treatment of larvae of the third stage of the same insect with extract Datura innoxia resulted in the failure of the resulting virility and death of pupae, At Allah [25] concluded that H. salicornica at a concentration 80 ppm caused mortality rate reached 41% for mosquito Culex pipiens pupae after 24 hour of treatment.

(	Concentration	Mortality %					
	mg/ml	E. obliqua	H.salicornicum	M. piperita			
	500	40.00Aa	33.33Ab	24.44Ac			
	300	33.33Bb	26.66Bb	13.33Bc			
	100	20.00Cc	16.66Cb	10.00Bc			
	Control	0.00Da	0.00Da	0.00Da			

### Table 3. Effect of phenolic extracts on pupa stage of M .domestica

L.D.S (0.05) = 3.408

Different capital letters denote to significant differences vertically ( among concentrations), Different small letters denote to significant difference horizontally Effect of phenolic extracts on the severity of the adults **4** -

The results of table (4) showed that phenolic extracts significantly affected the age of adults produced from larvae treated with concentrations of extracts when compared to the control agent, The results of the statistical analysis showed that there were significant differences between the extracts during the lifetime of adults. The treated males were able to stay for (11.80, 11. 90, 12.1) days for the adult from the larvae treated with the extracts of E obliqua., H. salicornicum , M. piperita compared to the control treatment 17.8 days . The females of the larvae treated with extracts reached (11.5, 12.2, 12.5) days compared to the control treatment 18.1 days , Koul [26] said the treatment of domestic fly larvae with E.globula extract reduced the age of the adult stage to 11.80 days.Zimmer [27] said that the period of growth of the adult role of domestic fly decreased to 12.17 days because of treatment with concentrations of 3.2 mg / ml of Neem plant extract.

Phenolic extract	Mortality %			
	Male	Female		
E. obliqua	11.80Aa	11.5Aa		
H. salicornicum	11.90Aa	12.2Aa		
M. piperita	12.1Aa	12.5Aa		
Control	17.8Ba	18.1Ba		
L.D.S (0.05) = 1.24				

Table 4. Effect of phenolic extracts in long age of adult stage at concentration (500 ppm)

Different capital letters denote to significant differences vertically ( among concentrations), Different small letters denote to significant difference horizontally The attractive and antagonizing effect of phenolic extracts in the adult role**5** -

Table (5) showed that all phenolic extracts showed an effect of repelling of domestic fly eggs with the highest expulsion rate of %80 for phenolic extract of E. obliqua plant, then the digestion extract by a package of %70 H.salicornicum and then the M. piperita %63.33.

Plants that have a repellent effect for adults can be used as sprayers and emulsifiers because they contain repellant substances which are anti-feeding in order to repel the insect from the source of food. This is consistent with Nassir [28], Who pointed out that the phenolic extract of peppermint extract caused a good expulsion of M. domestica fly

eggs , The results of the study by Xu and Zhang [29] in the knowledge of the effect of extruding and attractive to the extract of Evodia rutaecarpa in the flour beetle insect, the rate of expulsion was 56.66% after 20 minutes of treatment and the extract of the leaves gave a 70 % expulsion rate of domestic flies [30].

Concentration	Mortality %					
ppm	E. obl	iqua	H. salico	ornicum	M. piperita	
	plus	Neg	plus	neg	plus	neg
500	80.00Aa	0.00Ab	70.00Ac	0.00Ab	63.33Bd	0.00Ab
300	73.33Ba	0.00Ab	63.33Bc	0.00Ab	56.66Bd	0.00Ab
100	63.33Ab	0.00Ab	50.00Cc	0.00Ab	40.00Cd	0.00Ab
control	0.00Da	0.00Aa	0.00Da	0.00Aa	0.00Da	0.00Da

Table (5) Effect of phenolic extracts in plus of adults of M .domestica

# L.D.S (0.05) = 3.785

Different capital letters denote to significant differences vertically ( among concentrations), Different small letters denote to significant difference horizontally 6: Vitale assessment on bacteria

# 6-1 Isolating bacteria

The study included isolating bacteria from different environmental sources in Al-Diwaniyah city , the results showed the presence of 4 samples that had negative growth for bacterial culture , and 36 samples that had positive growth , 90% of which belonged to the type B. sphaericus , 80% belonged to the type B.subtilius , 74.3% belonged to B.lentimorbus and 66.6% to B.poppiliae and was diagnosed by biochemical methods by growing it on a sodium selective medium , and it was shown that thes bacteria produce exotoxin in the form of protein crystals when stained with coomassie brilliant blue .

#### Table 6. types isolating bacteria and their impression rate

Isolating Bacteria	Impression rate%
1- B. sphaericus	90.00
2 - B.subtilius	80.00
3- B.lentimorbus	74.33
4- B.poppiliae	66.66

#### Effect of phenolic extracts in bacteria

The results did not give any effect of phenolic plant extract of E. oblique , H.salicornicum and M. piperita on any type of isolated bacteria used at the concentration of 100 ppm in (Disc diffusion) test method. These results were consistent with the Bagci and Digrat [31] study, which indicated that the olive extract did not produce any effect against B. subtilius, And that the extract of the plant Eucalyptus sp. did not give any significant effectiveness in the direction of Bacillus bacteria [32] , Kuramman et al,[33] extracted a plant extract Juniperus oxyceedrus with 143 strains of bacteria. He found that the extract did not produce any effect in the direction of the bacteria of B.sphaericus at the concentrations of 25-250  $\mu$ g / ml. [34] also mentioned that the M.communis extract did not give any effect to the sex of Bacillus.

From the above which have showed to us that the possibility of using insect pathogenic bacteria with plant extracts within the framework of integrated pest control and to prevent the occurrence of pest resistance or delay the appearance and reduce environmental pollution. These results are consistent with the evidence of Linquist [35] that mixing the spores of bacteria B.sphaericus with plant extracts delayed the appearance of resistance in the mosquito Aedes aegypti.

Effect of phenolic extracts on fungi

The test for phenolic extracts showed a different effect on the fungus species used in the study at 100 ppm concentration and in the toxic food test method table (7). The statistical analysis showed significant differences the rate of inhibition of types fungi , the highest rate of inhibition was 21.2 % to the fungus Pethiumaltimum when using the extract E. obliquae , Vadya [36] pointed out that Eucalyptus extract has an inhibitory effect on the growth of fungi. When examining the sensitivity of Aspergillusflavus for plant extracts. Casentin [37] noted that the Junperus turbianate extract at a concentration of 90  $\mu$ g / mL gave a suppressive effect. In the study conducted by Ronuald et al, [38] found that the extract of Mentha piperita at a concentration of 2.5  $\mu$ g / ml recorded a rate of inhibition of 52 % for the growth of Trichoderma harzanium.. The phenolic extract of E.camaldulenisis has a high inhibitory effect against A. niger [39]. The effects of extracts on the growth of fungi may be due to their effect on the fungal cell membrane, which causes increased membrane maturity.

Fungi	inhibition				
	E. obliqua	H.salicornicum	M. piperita		
1- Isaria farinosus	1.3Aa	1.1Aa	0.00Aa		
2- Entomophthora schizophorae	5.8Ba	4.2Ba	1.3BB		
3- Verticillium fusisporium	11.1Ca	5.8Cb	3.1BCc		
4- paecilomyces fumosoroseus	13.7Da	9.1Db	4.3Cc		
5- Entomophthora muscae	12.6CDa	4.5Bb	2.2Bc		
6- Pethium altimum	21.2Ea	8.7Db	4.5Cc		
Control	0.00Fa	0.00Ea	0.00Aa		

Table 7. Effect of phenolic extracts in inhibition of some Fungi at concentration ofM.domestica

# L.D.S (0.05) = 2.08

Different capital letters denote to significant differences vertically , Different small letters denote to significant difference horizontally

# 4. Conclusions

The current study revealed that plant extracts are needed to help reduce the application of chemical insecticides, it can be very effective in certain conditions as demonstrated by elimination of M. domestica, the phenolic extracts of E. oblique, H.salicornicum and M. piperita have been extensively used due to its ability to selectively kill different life stages of M.domestica. So far larger number of study needed to determine if plant extracts can be an effective method against B. subtillus, B. sphaericus, B. lentimorbus, B. poppiliae and number of entomopathogenic fungi, the possibility of using mixtures of plants extracts with entomopathogenic bacteria preparations in the control of various insect pests, M.domestica play important role in vector control programs.

#### 5. Acknowledgements

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