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Leg Paralysis Inducement by Risella 17 Oil in *Lucilia sericata* (Meig) as Affected by Sex, Time, Application Site, and oil Volume[1]

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Key words: Risella 17 Oil; *Lucilia sericata* **(Meig); Leg Paralytic Induction, England.**

RINGKASAN

Kejadian kaki lumpuh yang disebabkan oleh 'Risella-17-oil' ke atas Lucilia sericata *(Meig.) mempunyai kaitan yang bererti dengan jantina, masa, bahagian anggota yang dirawat dan isipadu 'oil' yang digunakan. Adalah diperhatikan bahawa 'Risella-17-oil' memberikan kesan yang lebih nyata ke atas lalat jantan dibandingkan dengan lalat betina. Walaubagaimanapun, bagi kedua-dua jenis lalat, sama ada jantan atau betina, kejadian kaki lumpuh bertambah apabila isipadu 'oil' digunakan dengan berlebihan* PV_{50} *(isipadu 'oil' yang diperlukan bagi menyebabkan tanda-tanda lumpuh ke atas 50% lalat-lalat yang diberi rawatan) ialah 0.13 µl bagi lalat-lalat jantan dan 0.2 µl bagi lalat-lalat betina. Garis-garis regressi ialah* $y = 4.01\ x + 0.57$ *bagi lalat jantan dan* $y = 2.64\ x + 1.53$ *bagi lalat betina.*

Jumlah lalat jantan yang terkena kaki lumpuh adalah bergantung kepada bahagian anggota yang dirawat. Dengan menggunakan 0.3 µl 'oil', jumlah lalat yang terkena kaki lumpuh adalah dua kali ganda banyaknya bagi rawatan kepada abdomen dibandingkan dengan rawatan yang dilakukan ke atas thorax. Walaubagaimanapun tiada perbezaan yang bererti dapat dilihat di antara rawatan yang dibuat kepada bahagian belakang ataupun ke atas bahagian pedadaan abdomen. Lalat-lalat yang menerima rawatan di bahagian kepala dan kaki tidak menunjukkan tanda-tanda lumpuh. Setakat ini, penemuan-penemuan yang diperolehi dari penyelidikan mencadangkan bahawa 'Risella-17-oil' boleh membawa lebih daripada satu akibat. Namun begitu, kepentingan dan peranannya dalam menyebabkan berlakunya lumpuh kaki serta diikuti dengan kematian adalah memerlukan penyiasatan yang lanjut dan lebih kritikal.

SUMMARY

Development of leg paralysis induced by Risella 17 oil on Lucilia sericata *(Meig.) was found to be significantly affected by sex, time, application site, and oil volume. Male flies were noted to be more susceptible than female flies. In both sexes, more were affected at a given time when a greater volume of oil was applied. The* PV_{50} *(volume of oil required to induce paralytic symptoms in 50% of the treated flies) was found to be 0.13 µl for the male flies, and 0.2 µl for the female flies. Their probit regression lines are :* $y = 4.01x + 0.57$ *for the male, and* $y = 2.64x + 1.35$ *for the female.*

The number of male flies that developed leg paralysis was dependent on the site of application. With 0.3 µl of oil, there were about twice as many flies affected in treatment on the abdomen as compared to that on the thorax. However, no significant difference was observed between applications made dorsally and ventrally on the abdomen. Flies treated on the head and legs did not develop any paralytic symptoms. The findings suggest that there is more than one mode of action. However, the relative importance and role of any mode of action in contributing to the paralysis inducement with subsequent death, would require further and more critical investigations.

INTRODUCTION

Risella 17 oil is commonly used in insecticide formulations. In blowfly studies, it has been used alone or with insecticides; the treatments including varying dosages applied topically, by injections, or as surface residues (Bard, 1961; Lewis, 1962, 1963; Busvine, 1962, 1971). Although no inducement of leg paralysis was noted in these investigations, the oil was later

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found to induce leg paralysis with the blowfly, *Lucilia sericata* (Meig.) (Lim, 1972, 1976). Further studies were conducted to investigate the effect of the oil, particularly on the pattern of paralytic development (Lim, in press). However, the present investigations on paralytic inducement as affected by sex, time, application site, and oil volume, were aimed at providing deeper insights into their nature and relationships, well as a better understanding of some aspects on the mortality process since the paralysis is noted to be an extended state of moribund condition (Lim, 1972; 1976).

MATERIALS AND METHODS

Throughout the studies, the temperature was maintained at 26 ± 1°C and the relative humidity 65 ± 5%. All the flies used were unmated and of the same age (4 days old) and brood. Within 24 hours of emergence, the males were segregated on the basis of the distance between the eyes, which in the female is approximately more than one-third the total width of the head (Aubertin, 1933). This early separation enabled the flies to remain unmated and their age known throughout the studies.

During treatment the flies were temporarily immobilized by chilling. Application was made with a foot-operated Burkard micro-applicator fitted with an ordinary 1 ml glass syringe that carried a bent needle with a blunt tip. Each fly was topically applied with a nominal dose of 0.3 l as delivered by the applicator. Except for flies used in studies concerning sites of application, the treatment was on the anterior margin of the second last abdominal segment and on the ventral surface. Flies that were deformed or crippled by the necessary handling were destroyed and replaced.

After treatment, the flies were kept in round plastic containers (10 cm diameter × 3.7 cm high) in groups of ten flies/container. Water and food in the form of granulated sugar were provided. At regular intervals, the flies were observed for symptoms of paralysis; each fly was considered paralytic when it had at least two legs paralysed.

For each set of study, a parallel group of untreated flies was used as control check.

RESULTS

1. *Fly number affected in relation to time from application*

The number of flies affected by Risella 17 oil was observed to vary with the time interval from application (Fig. 1). For a particular volume of the oil applied, the number affected increased with time. Depending on the volume used and the sex of the treated flies, the effect may be exhibited as early as two hours after treatment. From then on, the number affected increased rapidly; the maximum (or limiting) number was reached by the second or third day after treatment when male flies were topically dosed with 0.2 and 0.3 μl of oil. No significant increase in the number of affected flies was observed after this time. Any fly that did not show any symptom then remained unaffected.

2. *Fly number affected in relation to sex*

The male and female flies showed differing susceptibility to the paralytic effects induced by Risella 17 oil. With a given volume, the male was observed to be more susceptible, generally having a higher maximum number of affected individuals (Fig. 1). Also, the time taken to reach the limiting level was faster. This was probably related to the different body weight and size of the two sexes, the male being much smaller (mean weight ± S.E. = 21.64 ± 0.99 mg) than the female (mean weight ± S.E. = 30.72 ± 1.17 mg).

3. *Fly number affected in relation to the volume applied*

The maximum number of flies affected was noted to depend on the volume of Risella 17 oil applied. In general, it increased with higher volume. For instance, with 0.2 μl and 0.1 μl of oil, the male flies showed a maximum of about 90% and 36% affected, respectively (Fig. 1). This general relationship was also observed in the female flies, although the rate of development and maximum number affected were comparatively lower for any given volume studied.

The volume of oil required to induce paralytic symptoms in 50% of the treated flies (PV_{50}) was determined for both the sexes. The regression lines obtained (Fig. 2) showed that in both the sexes, a linear relationship existed between the volume of the oil applied (log x) and the percentage suffering leg paralysis (probit y). Their regression equation are: $y = 4.01x + 0.57$ for the male, and $y = 2.64x + 1.53$ for the female. In the latter, the PV_{50} value is 0.2 μl with 95% fiducial limits of 0.11 μl to 0.37 μl. This is almost double that of the male which has a PV_{50} value of 0.13 μl with 95% fiducial limits of 0.08 μl to 0.19 μl. Evidently, the difference is related to the body weight since the PV_{50} for both sexes are almost the same when expressed against the weight, 0.0060 μl/mg for the male and 0.0066 μl/mg for the female.

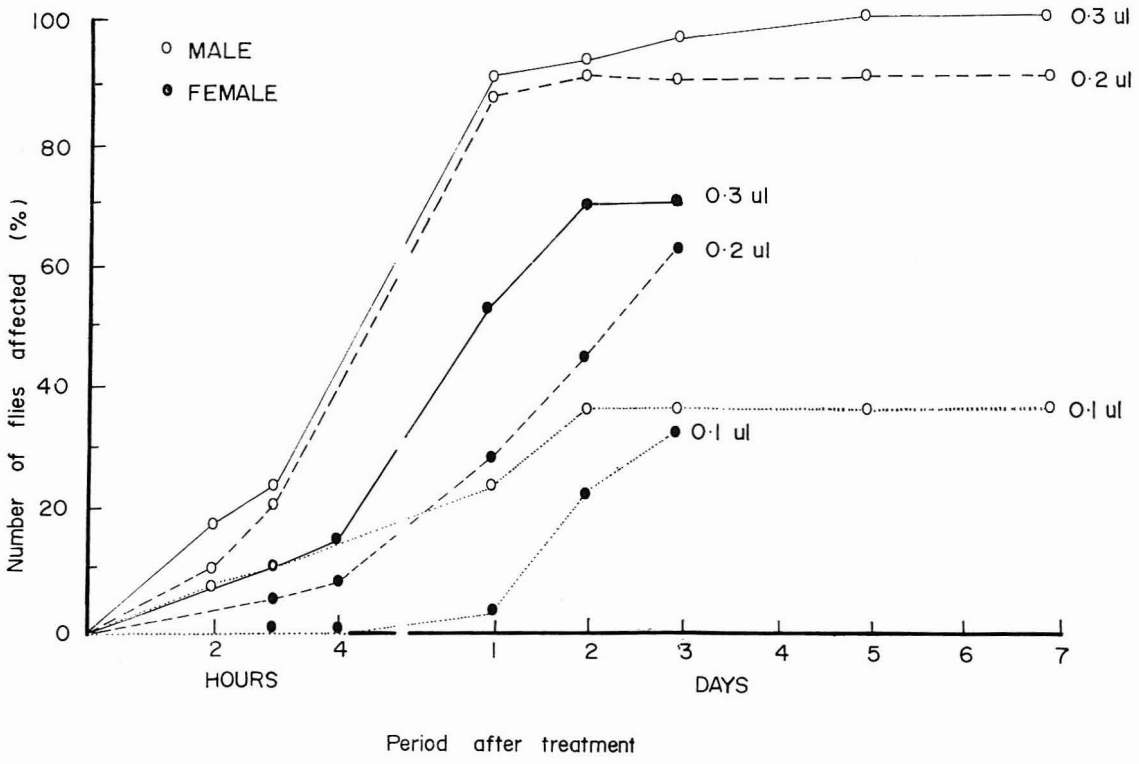

Figure 1. Number of Lucilia sericata *developing leg paralysis in relation to the volume of Risella 17 oil applied, time interval from application, and the sex of the flies. (Number of test flies/treatment: male = 30 – 40, female = 40 – 41)*

4. *Fly number affected in relation to the site of application*

In this study, male flies were randomly divided into 5 treatment groups. One of the groups was used as an untreated control while the remaining flies were treated topically with 0.3 μl Risella 17 oil according to their treatment groups which were as follows: dorsally on the head, dorsally on the thorax, dorsally on the abdomen, and ventrally on the abdomen.

The results showed that flies treated on the abdomen were about twice as susceptible than those which had applications on the thorax (Fig. 3). Among those treated on the abdomen, no significant difference was observed between those treated dorsally or ventrally. Throughout the study, neither control flies nor flies treated on the head appeared to be affected.

5. *Risella 17 oil treatment and fly mortality*

This study on the toxic effect of Risella 17 oil (applied at 0.3 μl) in relation to insect mortality, is based on the pooled data obtained from the various experiments conducted. A total of 200 treated male flies and 258 untreated male flies were studied.

As noted in previous studies, leg paralysis in treated flies occurred within two hours of treatment, increasing rapidly with time as was noted in more than 80% of the flies by two days following treatment. This pattern of development was observed, irrespective of whether the percentage paralysis calculated was based on only the surviving individuals, or with the dead individuals included under paralysis (Fig. 4).

In the study, a significant number of treated flies was observed to die following paralytic symptoms. This mortality was also noted to increase with time, reaching 66% at 7 days after treatment as compared to 13% in the untreated control (Fig. 4). Mortality began to set in around 24 hours after treatment. The significantly higher mortality observed suggested that the applied oil, under the conditions studied, had some toxic effect on the insect.

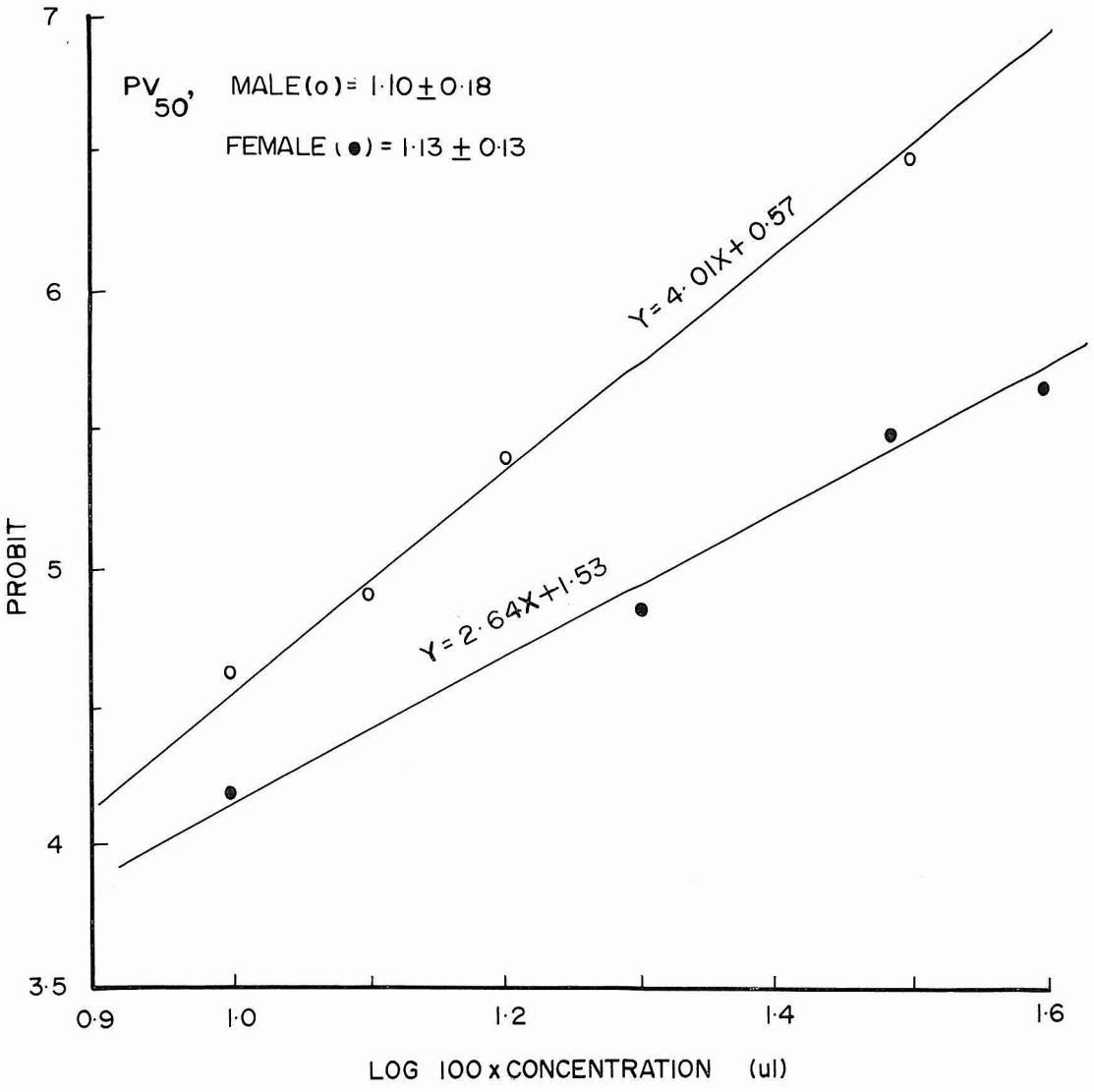

Figure 2. Regression lines for the calculation of the PV_{50} (volume of Risella 17 oil which induced leg paralysis in 50% of the treated flies), for both male and female Lucilia sericata *(Meig.). The oil was applied topically. (Number of test flies/concentration : male = 30–40, female = 40–41).*

DISCUSSION

From the investigations, it was evident that the number of flies affected by Risella 17 oil is closely associated with the time from exposure, the volume of oil to which flies were exposed, the site of application, and the sex of the flies. The males were noted to be more susceptible; in both sexes generally, the effect became more pronounced with increasing volume of oil applied. At high volume, the oil showed toxic effect.

Presently, the mode of action of the oil in inducing leg paralysis and subsequent death (for some) is still unclear. The findings suggested that the oil could act through the spiracles by

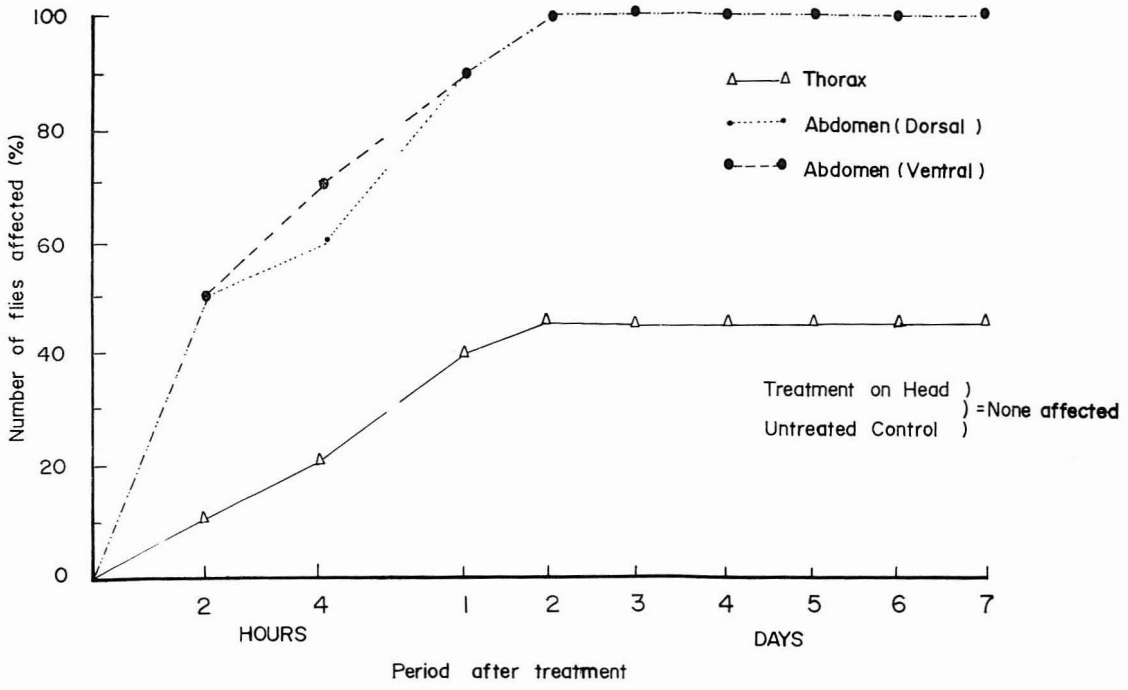

Figure 3. *Number of* Lucilia sericata *(Meig.) developing leg paralysis in relation to the site of application of the Risella 17 oil. (Treatment: topically with 0.3 µl; Number of test flies/treatment: 30–35 males.)*

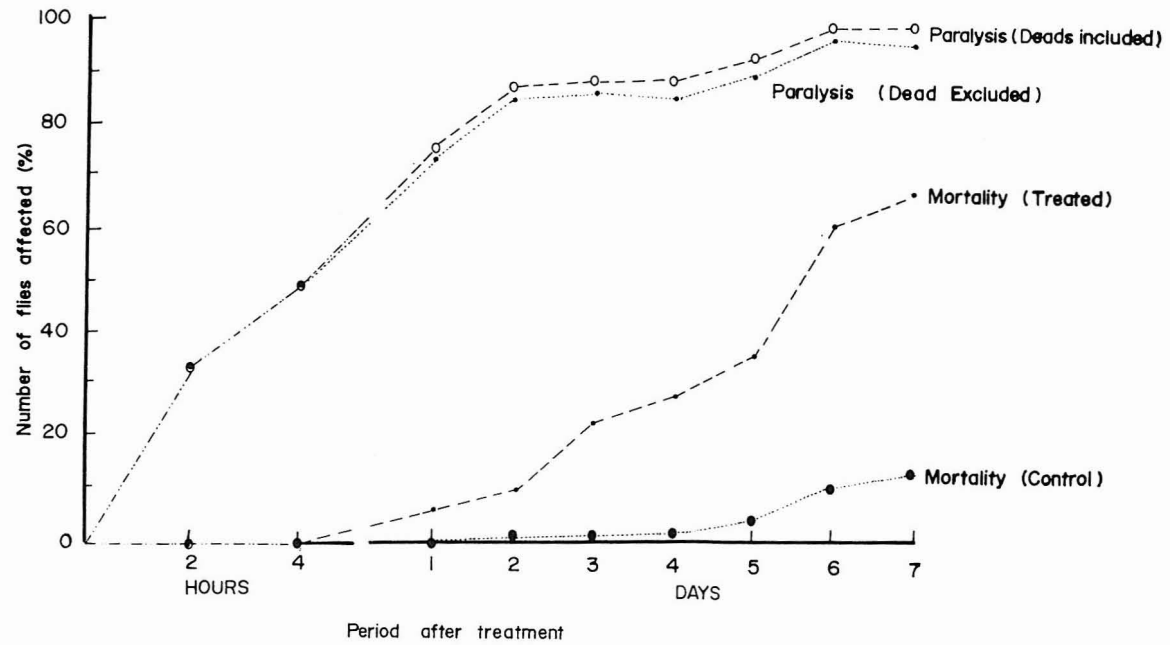

Figure 4. *Effect of Risella 17 oil, topically applied at 0.3 µl/fly, on the leg paralysis and morality of the male* Lucilia sericata *(Meig.)*

either physically blocking them and affecting the oxygen supply, or by entering rapidly into the body and to the vital organs through the spiracles and tracheal system. Whichever the route the mechanism of the paralytic induction is basically related to the nervous system. From the present studies, it was found that entry through the spiracles had special significance since flies treated on the head, where there were no spiracles, did not exhibit any paralytic symptoms; and thoracic treatments showed less effect than treatments on the abdomen which has many more pairs of spiracles.

Alternatively, the oil may dissolve and penetrate directly into the body through the cuticular layer. Such a mode of entry by chemicals has been reported or implied in reviews on this subject by several workers (Brown, 1951; Richards, 1951; 1953; O'Brien, 1967; Wigglesworth, 1942; 1948; and Ebeling, 1964). Based on this concept, the greater effect noted in the abdominal over the thoracic treatment may be explained by the greater surface area in the former. This allows the oil, which spreads readily on application, to act more effectively. However, since no paralytic symptoms were exhibited when treatment was made on the head, it would appear that the vital site of entry which resulted in leg paralysis, does not lie in the head. Otherwise, any direct penetration of the oil through the head cuticle should eventually also result in leg paralysis, unless differential cuticular penetration exists (Lewis, 1965), with little or no penetration taking place through the head.

The possibility of the leg paralysis being induced by the oil from purely physical effects on the legs directly was checked by treating flies on the legs only. Flies were treated with 0.3 μl of oil, either on the forelegs, midlegs, or hindlegs. In this study, the possibility that some of the oil applied on the legs may eventually creep up onto the body (Lewis, 1962) and induce leg paralysis in a manner similar to treatments that were made directly on the thorax or abdomen was considered. However, since no leg paralysis developed with such treatment, the cause of leg paralysis purely from the physical effects of the oil on the legs would thus appear to be unlikely.

The findings of this study suggest that there is more than one mode of action. However, the relative importance and role of any one mode of action in contributing to the paralysis inducement with subsequent death, would require further and more critical investigations.

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