



Pharmacological Research

The effect of *Emblica officinalis* diet on lifespan, sexual behavior, and fitness characters in *Drosophila melanogaster*

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Abstract

Drosophila is an excellent organism to test Ayurvedic medicines. The objective of our study was to explore the potential of *Emblica officinalis* drug on longevity, sexual behavior, and reproductive fitness of *Drosophila melanogaster* using adult feeding method. Increase in the lifespan, fecundity, fertility, ovarioles number, and developmental time was observed in both parents and F1 generation, but not in the F2 generation in experimental culture (control + *E. officinalis*). According to the Duncan's multiple range test and ANOVA, there is a significant difference between two cultures. It was also noticed that *E. officinalis* influence some fitness characters in *Drosophila* along with sexual behavior.

Key words: Ayurveda, *Drosophila*, *Emblica officinalis*

Introduction

Ayurveda literally "Science of Life"- is based on the twin principles of wholeness and balance. As a holistic healing tradition, Ayurveda recommends treating the "whole" person-body, mind, senses, emotions, and spirit instead of following the "one-cause-one-cure" principle and focusing on the symptoms of the moment. The premise on which this assumption is made is that if the roots are nourished and watered, the plant will flourish. Diet, sleep, lifestyle, daily and seasonal routines, and internal cleansing are just as, or more, important than herbs and potions in order to maintain good health. Ayurveda is equally about maintaining or preserving good health as it is about treating disorders--"Swaasthasya Rakshanam"(Protection of health) is one of the goal of Ayurveda.^[1]

The branch of *rasayana* or rejuvenation is one of the eight specialized branches of Ayurveda that primarily deals with the maintenance of health.^[2] *Rasayana* is defined as any herb, food, or activity which confers youthfulness and cures diseases. If taken in a proper way, the *rasayana* prevents early aging and keeps person young and active, both physically and mentally.^[3] The literal meaning of *rasayana* is "augmentation of *rasa*," the vital fluid produced by the digestion of food. *Rasa* provides nutrition, enhances the immunity, and sustains life. *Rasayana* is

the method of treatment through which the *rasa* is maintained in the body. The purpose of *rasayana* is to give strength, immunity, *ojus*, vitality, will power, and determination, and to strengthen the sense faculties^[4] so that you are not exposed to sickness and disease as long as you live.

According to *Sushruta*, sexuality and reproduction are so vital in Ayurveda that an entire discipline, known as *Vajikarana*, is dedicated to enhancing fertility and the rejuvenation of sexual and reproductive energy. *Vajikarana* therapy improves the function of the reproductive organs and vitalizes reproductive tissues, increasing semen count and strengthening their motility and making eggs more viable for conception. This not only enhances the quality and longevity of individual life, but also the health and vitality of offspring. Although some *Vajikarana* herbs work as aphrodisiacs, they also engender reproductive strength in order to increase the health of our offspring, or what *vajikarana* (aphrodisiacs) calls *subahupraja*.^[4] *Emblica officinalis* is such herb used as *rasayana* in treatment of diseases. However, effect of this drug on normal life activities has not been significantly validated. Therefore, the present study was carried out with an *objective* to explore the potential of *E. officinalis* drug on fitness of *Drosophila melanogaster* using adult feeding method. We have *D. melanogaster* as the test system which proved to be an excellent organism to test the effect of many drugs and other chemicals.^[5]

The fruit fly *Drosophila* is one of the most intensively studied in medicine and serves as a model system for investigations of many developmental and cellular processes common to higher eukaryotes, including man. This fly is being used for genetic studies since almost a century. Studies on *Drosophila* have enabled biologists to make significant contributions to the fields

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as diverse as basic genetics, population biology, evolutionary biology, behavioral biology, and molecular biology. It has a short life cycle, easy and inexpensive in culturing, and they could be kept in large numbers. Library of several *Drosophila* species with mutant and transgenic stock is available at different laboratories in the world. Presence of giant polytene chromosome and less number of the chromosomes are added advantages of the flies for genetic studies. Its genome has also been sequenced recently.^[6] Vertebrates have about four homologous for every gene found in *Drosophila*. The species shares large numbers of homologous genes with mammals, 13 601 with human beings. These have been analyzed to identify sequences related to those causing human diseases.^[7] The evolutionary conservation of gene function between human beings and *Drosophila* make it an ideal model system for the study of the molecular mechanisms of human disease. Moreover, identification of specific genes that regulate life span in *D. melanogaster* has been achieved by two processes: I. mutational analysis,^[8] in which manipulation of pathway function or gene has demonstrated life span extension, and II. Quantitative trait locus analysis,^[9] in which genetic elements affecting natural variations in longevity have been mapped to specific positions along the chromosomes.^[10,11] In *Drosophila* and many other insects, body size is positively correlated with mating success, longevity, fecundity, and other fitness characters and best phenotypic heritable characters.^[12] All these findings demonstrated the advantage of size in mating success and fitness. Various workers demonstrated the fitness studies in *Drosophila* such as fecundity, fertility,^[13] and longevity.^[14,15]

Survey of the literature shows^[11] on decrease gene activity such as *chico*, *dliip genes*, *dS6K*, *dTOR*, *DTS-3*, *EcR*, *Indy*, *InR*, *mtH*, *ovo*, *Puc* extends the lifespan in *D. melanogaster*. Simultaneously, there are genes such as *Cat*, *CcTl*, *dFOXO*, *DPOSH*, *dTSc1*, *dTSc2*, *EF-1a*, *fwd*, *G6PD*, *magu*, *Mel-41*, *Pemt*, and *PTEN* which increase gene activity leads to lifespan extends. Moreover, with these genes (*Catsup*, *Ddc*, *Dox-A2*, *Lim 3*, *stc*), allelic variation also varies longevity of fruit flies. These facts have shown that *D. melanogaster* is one of the most excellent eukaryote for exploring the genetic determination of life span, genetics of degeneration and aging process, and reproductive capacity. With these results, there is an insight into what to seek in man. For instant, the human *Xist* gene and the analogous *Drosophila Sxl* gene both control sex determination, and may both be involved in regulating longevity. This shows that any investigation of *E. officinalis* on the aging process on *Drosophila* will be fruitful research. In order to validate *E. officinalis* concepts more precisely, we proposed using *D. melanogaster* as a model. Till now, not much literature is available for evaluation of Ayurvedic medicine on *life span* on *Drosophila*. *E. officinalis* fruit is one of the Ayurvedic drug used in *Rasayana Chikitsa*. *Ayurveda* lexicons state that the fruit of *E. officinalis* is the best medicine to increase the lifespan. The biological effect of this drug has not been tested in recent times, especially in *Drosophila*, but only is being prescribed to human beings. Furthermore, assessing its effect in human beings has practical and ethical problems. Therefore, to revalidate the concept of *Rasayana Chikitsa* and in particular to assess the effect of *E. officinalis*, the present study has been carried out using *D. melanogaster* as the test system. A *rasayana* from *E. officinalis* was formulated based on the traditional principles; this is carefully altered to reflect intrinsic differences between mammals and insects. Here, we tried to investigate the impact of

the *E. officinalis* on fitness parameters such as number of fertility, fecundity, ovarioles, and developmental time in *D. melanogaster* flies and in next generation along with sexual behavior.

Materials and Methods

Preparation of cultures

To study the impact of *E. officinalis* on some quantitative sexual behavior and fitness parameters, *D. melanogaster* (Mysore strain) flies were obtained from *Drosophila* stock center, Department of Zoology, Mysore, and were used in the present study. The pure culture of these flies was maintained under standard food medium.^[16] The effect of *E. officinalis* was studied by adult feeding method. For this purpose, the stocks of the flies were built up for five to six generation from isofemale line. The virgin females and bachelor males that emerged from the normal media were isolated under ether anesthesia within 3 hours of eclosion. They were transferred to 8 × 2.5 cm glass culture vials containing equal quantities of normal food media used for *Drosophila* culture (wheat cream agar medium--this medium was prepared by boiling 1 000 ml of distilled water along with 100 g of *jaggery* [sugar]. When *jaggery* was dissolved in it, 100 g of wheat powder [*soji* or *rava*] was added to the medium and then 10 g of agar agar and 7.5 ml of propionic acid [antifungal] were added gently). The medium was distributed to glass vials of 8 × 2.5 cm size. The mouth of the bottles/vials was kept closed with cotton. One day later, one or two drops of yeast solution were added to the food media. This medium was used after 24 hours. At every step, heat vials were used for preparing medium. This was to prevent outbreak of pests and diseases. Similarly, sterilized cotton was used to plug the vials. This culture is used as control culture. For experimental culture, 5 drops of *E. officinalis* was included to the above wheat cream agar medium and virgin females and bachelor males were transferred into this media and aged for 4 days. There was no dietary restriction for the flies in both control and experimental culture.

Experimental design

For longevity (life span), 25 virgin females and bachelor males from both cultures were used to test the longevity. For this purpose, each pairs were transferred to control culture and experimental culture. Daily, these flies were transferred to the fresh vials of both cultures until flies die from the day of eclosion. This is calculated in terms of number of days.

For analysis of sexual behavior patterns, 50 virgin females and fifty bachelor males that emerged from the normal media were isolated under ether anesthesia within 3 hours of eclosion and maintained them separately in both control and experimental culture for 4 days. Twenty-five flies of both the sex were used to study some courtship activities,^[17,18] such as mating latency, copulation duration. For observation of sexual behavior, a virgin female and bachelor male were introduced into an Elens-Wattiaux mating chamber (5 × 5 cm circular glass chamber with a lid to facilitate easy observation). Because maximum mating occurs during morning hours, observation was made between 7 and 11 a. m. Mating latency (time between introduction of males and females into mating chamber and initiation of copulation of each pair) and copulation duration (time between initiation and termination of copulation of each pair) were recorded. The terminologies are used as per

the description of Hegde and Krishnamurthy.^[18] A minimum of 25 pairs involving each isofemale line were observed for both culture groups. Similarly, mating latency and copulation duration was also observed in the F1 generation and F2 generation (fed with normal food media), which were generated from the above 25 mated pairs which are considered as parents.

The reproductive fitness parameters such as fecundity, fertility, ovarioles number, and developmental time were analyzed from mated parents and their two successive generations. For fecundity test, mated males were transferred to vials containing normal food media and allowed to lay eggs for 24 hours. After 24 hours, the flies were individually transferred to fresh vial containing food media. The number of eggs laid (fecundity) during the following ten days was scored using stereomicroscope for both control and experimental groups. Twenty-five replicates were maintained for each of the control and experiment studies. The fertility was measured by counting the number of the progeny produced by a single mated female. For testing fertility, each mated female was kept in an individual food vial for a period of one day and then transferred to a fresh food vial every day. Ten successive changes were made and the total number of flies that emerged from each vial was counted. Twenty-five replicates were maintained for each of the experimental and control under study. Data were pooled and the mean number of flies per female was calculated. Counting of ovarioles number was done by selecting the 25 virgin females separately and maintained in both control and experimental cultures and aged for 4 days. These virgin females were dissected for left

ovaries in saline and bundles of ovarioles were separated by fine needle and counted under a stereomicroscope. For analysis of developmental time, after emergence of the flies were counted every day from the first to last day of the eclosion. A minimum two pairs of virgin female and bachelor's male were selected from all 10 vials which are considered as F1 generation. Similarly, for F2 generation, flies were selected from the F1 generation. These were mated separately maintained in the separate vial to calculate their fecundity, fertility, developmental time, and ovarioles number too. All the culture was maintained under the laboratory condition at a temperature $24 \pm 2^\circ\text{C}$. One way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) was applied for all parameters using SPSS 10.5 software.

Results

The data on the longevity (lifespan) presented in Table 1 reflect longevity of *D. melanogaster* where the parent flies fed with experimental culture shows high lifespan with mean value 76.8 ± 40.6 than F1 generation (70.3 ± 15.3), F2 generation (57.3 ± 15.4) with F value = 147.36 and control one (41.9 ± 14.0 ; F1 generation (40.2 ± 12.0); F2 generation (42.0 ± 20.9) with F value = 27.2.

The data on sexual behavior and fitness parameters of *D. melanogaster* of two different cultures have been reported in Table 2. Sexual activities such as mating latency was highest in control culture than experimental culture (10.32 ± 0.72) and it

Table 1: The longevity of *Drosophila melanogaster*

Parameters	Culture	Parents (Min/Max)	F1 generation (Min/Max)	F2 generation (Min/Max)	F value
Longevity	Control	41.9 ± 14.0^a	40.2 ± 12.0^b	42.0 ± 20.9^a	27.2*
	Experimental	76.8 ± 40.6^a	70.3 ± 15.3^b	57.3 ± 15.4^c	147.3**

Values in Means \pm SE; values with same alphabet as superscript are non significant at 5% level according to DMRT. *P < 0.01; **P < 0.001

Table 2: Fitness characters of *Drosophila melanogaster*

Parameters	Culture	Parents	F1 generation	F2 generation
Copulation duration (in minutes)	Control	19.76 ± 0.62	18.40 ± 0.42	20.41 ± 0.76
	Experimental	28.64 ± 1.15	26.42 ± 1.17	23.32 ± 2.16
	F = value	30.45**	27.15**	25.16**
Mating latency (in seconds)	Control	10.32 ± 0.72	10.14 ± 0.82	9.14 ± 0.42
	Experimental	7.20 ± 0.85	7.45 ± 1.06	9.02 ± 1.46
	F = value	20.04**	18.16**	15.23**
Fecundity (in numbers)	Control	118.3 ± 1.43^a	116.3 ± 1.22^a	115.3 ± 1.43^a
	Experimental	131.1 ± 1.36^b	128.1 ± 1.21^b	121.1 ± 1.19^b
	F = value	24.85**	23.65**	20.51**
Fertility (in numbers)	Control	106.3 ± 2.48^a	104.2 ± 3.34	102.8 ± 1.03
	Experimental	122.9 ± 1.32^b	120.3 ± 1.47	113.4 ± 1.35
	F = value	16.73**	15.43**	13.04**
Developmental time (in numbers)	Control	11.65 ± 0.09^b	11.43 ± 0.11	10.02 ± 0.09
	Experimental	15.63 ± 0.15^a	14.04 ± 0.13	11.03 ± 0.13
	F = value	6.57*	5.06*	6.46*
Ovarioles number (in numbers)	Control	14.00 ± 0.13^a	13.00 ± 0.12	13.00 ± 0.10
	Experimental	17.00 ± 0.73^b	15.00 ± 0.71	12.00 ± 0.62
	F = value	15.75**	13.04**	14.04**

Values are Means \pm SE, Different alphabet as superscript in each column is significant by DMRT, *P < 0.01; **P < 0.001; Control + *Emblca officinalis* (experimental culture)

is significant by one way ANOVA and DMRT (F value = 20.04; $P < 0.001$) between the two cultures; this is also seen in their successive generations. Meanwhile, copulation duration is highest in experimental culture (28.64 ± 1.15) compared with control culture. This prolongation of copulation was also observed in the next generation.

The scrutiny of Table 2 shows that fitness parameters such as fecundity, fertility, developmental time, and ovarioles number was high in experimental culture compared with control and significant too between two cultures (fecundity = 131.1 ± 1.36 , fertility = 122.9 ± 1.32 , developmental time = 15.63 ± 0.15 , ovarioles number = 17.00 ± 0.73 , F value = 24.85; 16.73; 6.57; 15.75; these mean values are more compared with their F1 and F2 generations. Table 2 presents mean values of fitness parameters, where F1 progeny shows high values compared with F2 generation (Fecundity = 128.1 ± 1.21 , fertility = 120.3 ± 1.47 ; developmental time = 14.04 ± 0.13 ; ovarioles number = 15.00 ± 0.71). All these parameters are highly significant when comparison is done between the cultures [Table 2]. The above fitness parameters and sexual behavior are depicted in Figures 1 and 2, where flies reared in control media show no such variation among the parameters, but there is variation in the mean values in the experimental culture between the parents, F1 generation, and F2 generation.

Discussion

For the above results, the longevity is more in case of parents compared with their progenies in experimental culture-fed flies than control one; this confirms that there is an influence of *E. officinalis* which is mixed in experimental culture. These results validate the statement of Acharya Charaka which says “Amlakah vayah sthapananam sreshtha”^[19] (Fruit of *E. officinalis* is the best to increase the longevity). Sexually reproducing animals are endowed with special features, first to produce fertile offspring and second to adapt to a particular environment. The reproduction is preceded by a series of courtship acts wherein males and females show unique rituals to attract each other, mate, and produce the offspring. The courtship and mating although are genetic, are also influenced by various factors. This may imply that these courtship activities are directed by the same set of genes and that these traits are related genetically. This agrees with the observations which show the genetic determination of certain components

of sexual behavior in *Drosophila*.^[20-22] Though genetically determined, there is every possibility for a change in sexual behavior because these activities are also influenced by changes in physical environment.^[23-27] In the present study, an effort is made to study the effect of *E. officinalis* on quantitative sexual behavior and fitness parameters.

The data obtained from our studies show that the mating latency was shorter in the flies reared in experimental culture in comparison with control [Table 1]. The differences in mating latency in two cultures were also statistically significant (by ANOVA and DMRT). Mating latency is measured as the time taken for the male oriented toward female until initiation of copulation duration by Markow.^[28] It is the period during which the pairs acclimatize in the mating chamber and then start the courtship activities. It actually indicates the vigor of male.^[28] A male with high vigor reacts quickly in the presence of female, whereas a male with less vigor reacts slowly.^[29-30] Obviously shorter mating latency indicates higher vigor of male. The shorter mating latency was noticed in experimental culture. This suggests that the males from experimental culture have higher vigor and therefore are quickly attracted by the females. The mating latency was also shorter in the flies from experimental culture compared with control shown in Figures 1 and 2. The mating latency not only indicates vigor of males, but also receptivity of females. It is the time required for males and females to initiate copulation. Higher the vigor of males and receptivity of females, shorter is the mating latency. During this period, courtship acts are performed mostly by males, to increase the receptivity of females and to make her sexually excited.^[22] A male with high vigor has to perform the same courtship act more number of times to a nonreceptive female than to a receptive female. If she is receptive, only a few courtship acts are performed leading to quick pairing. The short mating latency of experimental culture in the present study indicates that the males maintain high vigor and females maintain high receptivity at this culture, while those in control could not maintain the vigor and receptivity. Mating latency of control culture of parents and their generations are more or less equal [Table 2].

Courtship activity of the male or female culminates in copulation demonstrated by Spiess.^[21] During copulation, sperms from the male is transferred to the female reproductive tract and therefore the duration of copulation has a lot of significance in an animal's life. In the present study, the copulation duration was longest in flies maintained on

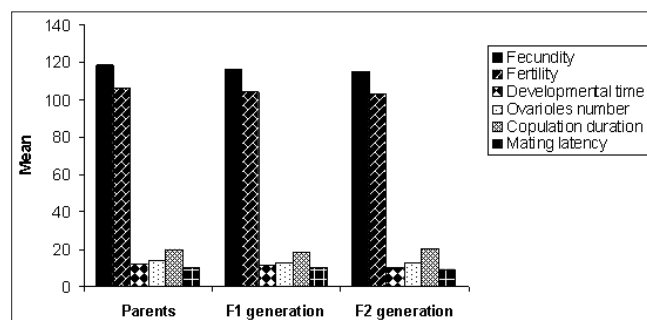


Figure 1: Fitness parameters and sexual behavior of *Drosophila melanogaster* cultured in control media (units; fecundity, fertility, developmental time, ovarioles = numbers, copulation duration = minutes; mating latency = seconds)

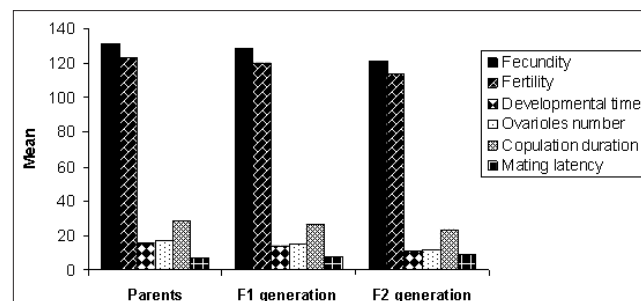


Figure 2: Fitness parameters and sexual behavior of *Drosophila melanogaster* cultured in experimental media (Control + *Emblica officinalis*) (units; fecundity, fertility, developmental time, ovarioles = numbers, copulation duration = minutes; mating latency = seconds)

experimental culture than those maintained on control culture. According to Guruprasad *et al.*,^[31] longer duration of copulation permits the transfer of more number of sperms by male to the female. Therefore, extension of copulation duration enhances the fitness of the male. It can also enhance the fitness of the females because the sperms received by a female can fertilize more number of eggs. Therefore, it is unlikely that the longer copulation duration could enhance the fitness, as the females have high receptivity and males have high vigor.

In *Drosophila*, the fecundity remains one of the less-known quantitative traits along with fitness parameters such as fertility, developmental time, and ovarioles number. Estimation of fecundity and fertility is important in routine testing of various chemicals. This gives an insight into the extent of effect on ovarioles and other physiological factors, which is expressed in the terms of egg and offspring production. Table 2 reveals that the mean fecundity eggs/female in the adult feeding methods was more than control. ANOVA and DMRT analyses have shown that increased fecundity in experimental culture reared flies were significant ($P < 0.001$). This indicates that the mode of administration is also important factor, which one should consider while assessing the effect of any chemical on any biological system. In contrast to this, in control culture, the decrease in fecundity may be accounted for the fact that the flies are under the influence of *E. officinalis*; hence, they might not have been able to lay eggs rather than producing less egg. This finding agrees with the observation^[32] where they noticed oviposition rhythm in *D. melanogaster*, and Vogel^[33] has demonstrated that certain aziridine analogs have discernible effect on fecundity in *Drosophila*. Table 1 also incorporates that mean fertility per female in adult experimental diet with *E. officinalis* significantly increases when compared with control, and they were more fertile than control, ($P < 0.001$ by ANOVA and DMRT). Several workers have made studies on the effect of different chemicals on fertility in *D. melanogaster*.^[34] The present study agrees with them that influence of the chemicals will alter the fertility in *Drosophila*.

Rate of development is another parameter, which is used to evaluate some chemicals clinically. In the present investigations, the genetic constitution, amount of the food, temperature, and space were kept constant. Obviously, the differences in the development must have been determined by the chemical used or not by the other factors. This type of effect on the developmental time by different chemicals in *D. melanogaster*.^[35] is well known.

The estimation of fitness is the first step in understanding the adaptive evolution of a population. Ovariole number is an anatomical trait determined during pupation for which a polygenic basis is known in various species of the *D. melanogaster* complex. Interestingly, our results are contrary to this where experimental culture-fed flies are having more ovarioles than control; this shows that there is influence of the *E. officinalis rasayana* mixed in experimental culture.

Figure 1 suggests that all the fitness parameters and sexual behavior of *D. melanogaster* reared in control media is similar in all parents and their generation too. According to Figure 2, fitness parameters and sexual behavior of *D. melanogaster* in experimental media have variation among F1 generation and F2 generation compared with their parents. The Flies which

are fed with the experimental culture have long life span than control one [Table 1]. Our results confirm the effect of *E. officinalis* on parents than their progenies and the impact of *E. officinalis* is carried to F1 generation rather to F2 generation in experimental culture.

Conclusion

The data generated in this study showed variation in the developmental time in both cultures [Table 2]; longest mean of developmental time was noticed in the experimental cultures. The conclusion of our experiment suggests that *E. officinalis* enhances the sexual activities. This obviously increases longevity, fertility, fecundity, ovarioles number along with developmental time. Finally, it is inferred that there is a linear interrelationship between sexual activities and fitness parameters in experimental culture. "Adding Life to years is better than adding years to life." So, along with longevity, other reproductive fitness characters of flies were considered for this study so as to explore the hidden principles of *Rasayana* therapy which improves the quality of the life.

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हिन्दी सारांश

आमलकी आहार का ड्रोसोफिला मेलानोगेस्टर की आयु और प्रजनन संस्थान पर प्रभाव

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आयुर्वेद में रसायन चिकित्सा का एक विशिष्ट स्थान है। यह रसायन प्रयोग वैसा ही जीवनप्रद है, जैसा देवताओं के लिये अमृत हितकर होता है। इसका प्रभाव अगम-अपार-अचिन्त्य और विलक्षण है। यह युवावस्था स्थापित करता है। आयुर्वेद में आमलक को एक श्रेष्ठ रसायन बताया गया है और कहा गया है "आमलकः वयः स्थापनानां श्रेष्ठः"। इस सिद्धान्त को स्थापित करने के लिये ड्रोसोफिला मेलानोगेस्टर को अनुसन्धान के लिये चुना गया। आमलकी चूर्ण का आयु, यौन व्यवहार, प्रजनन क्षमता, अण्डकोष क्षमता और विकास काल आदि मापदण्डों पर अनुसन्धान किया गया। इस अनुसन्धान से ये सुनिश्चित हुआ कि आमलकी चूर्ण के प्रभाव से आयु, यौन व्यवहार, प्रजनन क्षमता, अण्डकोष क्षमता और विकास काल में वृद्धि हुई और कन्ट्रोल ग्रुप की अपेक्षा महत्वपूर्ण अन्तर देखा गया।