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The virulence of the entomopathogenic fungi on the predatory species, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) under laboratory conditions

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Abstract

The objective of this study was to assess the survivorship and fecundity of the newly emerged females of the predatory species, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), exposed to LC_{50} of the entomopathogenic fungi (EPF); *Beauveria bassiana* and *Metarhizium anisopliae* under laboratory status. The virulence of the EPF was evaluated, using the dipping implementation technique. LC_{50} values were determined based on the concentration-response analysis. The LC_{50} value for the predator's females was 3×10^9 and 6×10^8 conidia/ml for *B. bassiana* and *M. anisopliae*, respectively. Life table and population parameters were the models to estimate survivorship of each stage of *C. montrouzieri*. Results demonstrated that the highest mortality rates 20.35, 27.40, and 29.45% occurred during the larval stage, whereas the total mortality rates attained 28.57, 38.61, and 44.66% for the control, *B. bassiana*, and *M. anisopliae*, respectively. For fecundity, the final proliferation average (R_0) of the population was 338.82, 155.99, and 115.55; mean generation time (T) was 43.76, 60.95, and 76.78; natural increase (r_m) was 0.13, 0.08, and 0.06; the steady rate of increase (λ) was 1.14, 1.08, and 1.06; and the required time to double the population (D_T) was 5.33, 8.66, and 11.55 days for the control, *B. bassiana*, and *M. anisopliae*, respectively.

Keywords: Entomopathogenic fungi, *Beauveria bassiana*, *Metarhizium anisopliae*, *Cryptolaemus montrouzieri*, Life table, Effect

Background

The coccinellid beetles occupied a major economic significance in agro-ecosystem, where they can be powerfully utilized in the biological control of numerous detrimental insects. The ladybird, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), is an active natural enemy of mealybugs that acting as a significant predator inside the IPM of mealybug pests in diverse cultivations. This beetle has been noted to prey on about 21 mealybug species (Jiang et al., 2009) and exceedingly to be applied as a biotic control factor in regions, where mealybugs such as *Planococcus citri* (Risso), *Maconellicoccus hirsutus* Green, *Phenacoccus solenopsis* Tinsley, and *Ferrisia virgata* outbreaks be found (Kaur and Virk, 2012).

Utilizing the entomopathogenic fungi (EPF), *Beauveria bassiana*, *Metarhizium anisopliae*, and *Verticillium lecanii* as biopesticides have been greatly applied for pest control (Faria and Wraight, 2007).

One of the substantial facts that should be taken into account in the utilization of EPF as biological control agents is their harmony with other biological control factors. In spite of some EPF are renowned, particularly some of those relate to Deuteromycotina, they have a rather vast range of hosts from numerous insect orders and natural enemies (Inglis et al., 2001). Integration between EPF and predators is needed to increase the role of bioagents and decrease the hazards to the environment.

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B. bassiana and *M. anisopliae* give rise into white and green muscardine disease in the enormous extent of insects and are presently utilized inside the greenhouse and orchard crops for the management of agricultural arthropod pests involving whiteflies, aphids, thrips, psyllids, mealybugs, and weevils (Thungrabeab et al., 2006). In the status of EPF, the fatal and sub-fatal influences of the pathogen on useful insects (predators and parasitoids) considering fertility, lifespan period, and survivorship are in need to be estimated. Life table investigations supply a potent method for amount estimation of naturalistic enemies to elaborate characterization age-specified mortality of individuals in inhabitants (Luck et al., 1988). When knowledge about the quality of insect fertility and mortality that occur at any age is obtainable, the influence of the naturalistic enemy is skillfully obvious on the one hand with its impact on measuring the pest population development rate (van Driesche and Bellows, 1996).

There is extremely slight information on the appropriate employment of *B. bassiana* and *M. anisopliae* on *C. montrouzieri* for integrated pest management of *Planococcus ficus* (Signoret) according to utilizing the life table technique.

Therefore, the target of this investigation is to estimate the impact of *B. bassiana* and *M. anisopliae* on *C. montrouzieri* via investigating the predator's biological parameters.

Materials and methods

Culture of prey and predator

The vine mealybug, *Planococcus ficus* (Signoret), was reared on pumpkins to gain a colony for the duration of work as the method described by Pang and Gordon (1986). Pumpkins were washed by water to remove dust and dried with tissues. The ovisacs, crawlers, and adults of the vine mealybug gained from infested grapevine plants at Qena Governorate, Egypt, and were transmitted onto the pumpkins. Infested pumpkins were preserved in a cylindrical plastic crate (30 cm diameter). They were maintained at a relative humidity of 45 ± 5%, a temperature of 25 ± 1 °C, and a 16:8 day time, darkness period. Subsequently, the numbers of vine mealybugs had an excess on pumpkins then adequate *C. montrouzieri* adults were released on the vine mealybugs. Adults and eggs of the predator that were utilized for the trials were acquired after completing one generation of rearing.

Applied fungi

Two species of the EPF, *B. bassiana and M. anisopliae*, were obtained from Assiut University Mycological Center (AUMC), Egypt. The two fungal species were preserved in a refrigerator pending utilizing them for conidiospores output.

Conidiospore production

The fungi *B. bassiana* and *M. anisopliae* were grown on a medium of Potato Dextrose Agar (PDA) which poured into Petri plates. A hemocytometer was applied to calculate the concentration of conidia in the stock suspension. After that, dilution was carried out to obtain the required concentrations of the conidia (Nazir et al., 2007).

Bioassay

Three concentrations of conidial suspension (5×10^7, 5×10^8, and 5×10^9 conidia/ml) from *B. bassiana* and *M. anisopliae* were prepared. All bioassay trials were executed in the laboratory at 25 ± 1 °C. Ten new emerged females of *C. montrouzieri* were handled by immersing them for 5 s in the conidial suspension in 50 ml conical flasks. After immersing inside the suspension, the insect predators were maintained by the vine mealybug into a Petri dish (9 × 1.5 cm). Thereafter, they were preserved for 7 days at 25 °C and 80–90% RH. A treatment with sterile distilled water + 0.05% Triton X-100 without fungal conidia was utilized as a control. Four duplicates for each treatment, containing 10 insects, were used. Lifeless insects were numbered next 1, 3, 5, and 7 days. Results were analyzed by probit analysis employing SPSS computer program to estimate LC_{50} values.

Effect of LC_{50} concentration on life table parameters of the treated unmated females of *C. montrouzieri*

Twenty-five recent female predators staying lively after 72 h from treating with the LC_{50} concentration of the fungi were applied for this experiment. Every single female was displayed on an untherapy male from the supplies settlement, singularly. The lifeless males were substituted with life males. In the whole testing, the diverse phases of the vine mealybug and *P. ficus* were extended like a nutrition exporter. The death rate and oviposition were registered every day till the demise of the final female in treatments and controls together. Life and fecundity tables were applied to estimate the influence of the LC_{50} concentrations of the fungi on pre-ovipositional, ovipositional, and post-ovipositional periods and daily fecundity and longevity of *C. montrouzieri*. Life and fecundity tables were the subsequent accomplishment of Southwood (1978). Life table coefficients were calculated as follows:

x: Age cohort in factors of time (days)/growing stage

l_x: Number of staying vivid individuals at the beginning of age category (x)

L_x: Number of individuals alive between age and the age period that followed

d_x: Number of death through age period x

100qx: Percent ostensible of the death-rate, 100qx = $(d_x/l_x)100$

S_x: Survival stage rate within the stage

T_x: The overall figure of age x units after the age x

ex: Life anticipated for individuals of age x, $e_x = T_x/l_x$

Age-specific fertility table was constructed with the following columns:

X: Actual female age (time from eggs).

m_x: age-specific fecundity, the number of living females born per female in each age period

R_o: The final propagation rates, $R_o = \Sigma\, l_x m_x$

T: Average generation period, $T = \ln R_o/r_m$

D_T: Twice the amount of time, the number of days required by a population to double, $D_T = \ln 2/r_m$

r_m: The instinctive ability for the augmentation, $r = \ln R_o/T$

λ: The limited average of augmentation, $\lambda = e^r$

Results and discussion

Dose–response bioassay

Results of the pathogenicity test showed that the treatments with the two entomopathogenic fungi reduced the number of predator females C. *montrouzieri*. The LC_{50} values and the slope of are shown in (Table 1).

The lowest LC_{50} value (6×10^8 conidia/ml) was recorded for *M. anisopliae* treatment. As stated about the LC_{50} values, *M. anisopliae* isolate possessed a great influence against the predator females, followed by *B. bassiana*. Ibrahim et al. (2011) reported that different isolates of *M. anisopliae* were generally virulent to C. *montrouzieri* predator. No death was recorded in the control. All concentrations that were used from the previous fungi had a potential effect on the female of C. *montrouzieri*. The mortality rate of the female depended on the species of fungi used and also on the dose concentration. The mortality rate at the end of the experiment, resulting from various concentrations of the EPF, is illustrated in (Fig. 1). The accumulative mortality of C. *montrouzieri* females displayed on isolates of the fungi extended from 25 and 52% to 32.5 and 65% for *B. bassiana* and *M. anisopliae*, respectively.

The study outcome is similar to the results of Thungrabeab and Tongma (2007) who found that *M. anisopliae* was more effective than *B. bassiana* against *Coccinella septempunctata* L. Also, Trizelia et al. (2017) noticed that up to (67.50%) mortality rate of different larval instars of the predator, *Menochilus sexmaculatus* Fabricius occurred by various strains of *M. anisopliae*. Similarly, Er et al. (2008) reported that *M. anisopliae* caused (47.61%) mortality rate to *C. septempunctata* adults.

A toxin known as destruxin, which is produced by *M. anisopliae* and *B. bassiana* through the infection was believed to be the reason of *C. montrouzieri* death. Destruction affected target cell organelles (mitochondria, endoplasmic reticulum, and nuclear membrane) and caused cells paralysis. It also affected the mesenteron, malphigian tubes, and tissue larval hemocyte disfunction (Tanada and Kaya, 1993).

There is a close relationship between the conidiospores concentrations of *B. bassiana* and *M. anisopliae* and the proportion of the noticed mortality. The minimum fungal concentration (5×10^7 conidia/ml) caused less death percentages (25 and 32.5%) for *C. montrouzieri* females.

Consequently, the weak potency of fungi could be due to the faint conidial concentrations, which the maximum concentration was 5×10^9 conidia/ml of the two tested fungi that brought out high mortality percentages of 52 and 65%, respectively. Scorsetti et al. (2012) compared the ability of *B. bassiana* and these concentrations on *Eriopis connexa* (Germar) predator of aphids (Hemiptera: Aphididae) and stated that all *E. connexa* stages utilized were sensitive to *B. bassiana*. Thungrabeab and Tongma (2007) found relationships between the virulence of *B. bassiana* and *M. anisopliae* and their concentrations on non-target insects.

Table 1 LC_{50} values of entomopathogenic fungi on the female of *Cryptolaemus montrouzieri*

Fungal isolates	LC_{50} conidia/ml	Confidence limits		Slope ± S.E
		Lower	Upper	
Beauveria bassiana	3×10^9	7×10^8	2×10^{12}	0.37 ± 0.15
Metarhizium anisopliae	6×10^8	1×10^8	3×10^9	0.41 ± 0.15

Effect of LC_{50} concentration values of *B. bassiana* and *M. anisopliae* on biological parameters and reproductive performance of the treated unmated females of C. *montrouzieri*

Age-specific survival life table

The percentage of mortality in egg stage registered (12.64, 15.05, and 6.98%) on emerged female individuals after treatment with LC_{50} concentration of EPF than in the control, respectively, due to the effect of EPF on physiological causes leading to infertility as well as their effect on genetic factors. In line with obtained outcomes, Nalepa and Weir (2007) explained that the percentage of mortality during the egg phase was 14.66 and 11.53%, when evaluating the effect of EPF, *M. anisopliae* and *Hesperomyces virescens* on the life table of the coccinellid, *Harmonia axyridis*.

The highest rates of mortality found in young larval instar and then began to decline gradually in the elderly instars (Tables 2, 3, and 4). The highest mortality rate was registered in the 1st instar larvae (10.10, 9.71, and

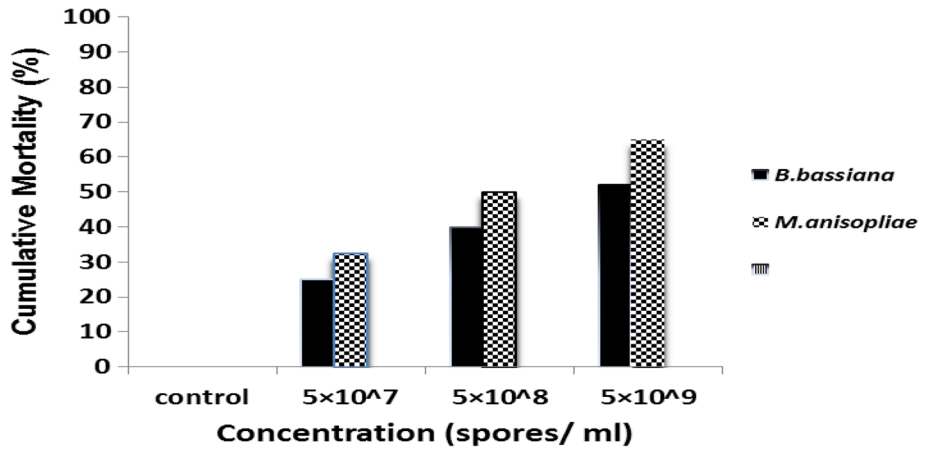

Fig. 1 Accumulative mortality (%) of *Cryptolaemus montrouzieri* adult

6.35), followed by 2nd instar larvae (8.84, 8.25, and 5.71), 3rd instar larvae (6.63, 2.91, and 3.49), and 4th instar larvae (6.25, 2.43, and 2.86) for female individuals, which remained alive 72 h after exposure to LC_{50} concentration of *B. bassiana* and *M. anisopliae* than in the control, respectively.

The larval stage registered the highest mortality rate due to the length of the larval life compared to the rest of the other stages. The mortality percentage of the larval stage registered (27.40, 29.45, and 20.35%) after treatment with *B. bassiana* and *M. anisopliae* than in the control, respectively.

The response of the first instar larvae to the effect of different fungus species was due to the heterogeneity of the cuticle layer at these instars. In contrast, the weak effect of different types of fungi on the adult stage of this insect may be due to the sclerotization and tanning of the cuticle layer that impeded the penetration of fungal hyphae. Thungrabeab and Tongma (2007) also suggested that the cuticle of *C. septempunctata* had the biggest influence on the penetration of *B. bassiana* and *M. anisopliae*.

Overall, data in Tables 2, 3, and 4 presented the total mortality rate of *C. montrouzieri* (38.61, 44.66, and 28.57%), when it is exposed to *B. bassiana*, *M. anisopliae*, and the control, respectively. The investigation moved in the identical line of Trizelia et al. (2017) who realized that the total percentage of the mortality of *Menochilus sexmaculatus* Fabricius ranged from 27.50 to 67.50% after treatment with *Metarhizium* spp. isolates.

Age-specific fecundity table

Age-specific survivorship (l_x) and fertility (m_x) of *C. montrouzieri* on resulted females remained alive after 72 h after exposing to LC_{50} concentration of *B. bassiana* and *M. anisopliae* and in the control are shown in Fig. 2.

The initial female emerged on the days 46, 54, and 33 and the precocious egg deposition was registered after 11, 18, and 7 days of female appearance and nearly continued until female death. While the first case of death was registered for adult females in 72, 91, and 61 days

Table 2 Life table of *Cryptolaemus montrouzieri* females treated with LC_{50} concentration of *Beauveria bassiana*

x	l_x	L_x	d_x	100_{qx}	s_x	T_x	e_x
Eggs	277	242.5	35	12.64	87.36	1043.5	3.77
1st instar	208	194.5	21	10.1	89.9	801	3.85
2nd instar	181	173.5	16	8.84	91.16	606.5	3.35
3rd instar	166	155	11	6.63	93.37	433	2.61
4th instar	144	131	9	6.25	93.75	278	1.93
Pupae	118	103	15	12.71	87.29	147	1.25
Adult	88	33					

Table 3 Life table of *Cryptolaemus montrouzieri* females treated with LC_{50} concentration of *Metarhizium anisopliae*

x	l_x	L_x	d_x	100_{qx}	s_x	T_x	e_x
Eggs	206	184.5	31	15.05	84.95	724	3.51
1st instar	163	139	20	9.71	90.29	518	3.18
2nd instar	115	100.5	17	8.25	91.75	355	3.09
3rd instar	86	76	6	2.91	97.09	240	2.79
4th instar	66	58	5	2.43	97.57	154	2.33
Pupae	50	44	13	6.31	93.69	88	1.76
Adults	38	19					

Table 4 Life table of *Cryptolaemus montrouzieri* females under control treatment

x	l_x	L_x	d_x	100_{qx}	s_x	T_x	e_x
Eggs	315	300	22	6.98	93.02	1598.5	5.07
1st instar	285	273	20	6.35	93.65	1298.5	4.56
2nd instar	261	254.5	18	5.71	94.29	1025.5	3.91
3rd instar	248	240	11	3.49	96.51	771	3.11
4th instar	232	225	9	2.86	97.14	531	2.29
Pupae	218	207.5	10	3.17	96.83	306	1.4
Adults	197	98.5					

on emerged females after treatment with LC_{50} concentration of *B. bassiana*, *M. anisopliae*, and distilled water (control), respectively.

The latter female endears on days 77, 94, and 64. Moreover, results in Table 5 demonstrated that the pre-ovipositional period lasted about 10.13, 17.13, and 6.23 days; ovipositional period lasted about 15.14, 19.21, and 21.13 days; and post-ovipositional period lasted about 5.14, 2.18, and 3.22 days for female after treatment with *B. bassiana*, *M. anisopliae*, and in the control. The results obtained from Tables 2 to 5 had also supported that the virulence of *M. anisopliae* was higher than *B. bassiana* on fecundity, pre-ovipositional, ovipositional, post-ovipositional periods, and longevity of *C. montrouzieri*.

Numerous researchers recorded that the fertility, pre-ovipositional, ovipositional, post-ovipositional periods, and life span of *C. montrouzieri* predator were impacted by EPF. Ibrahim et al. (2011) mentioned that *M. anisopliae* impacted the pre-ovipositional, ovipositional, and post-ovipositional periods of *C. montrouzieri*. Also, Ana et al. (2017) stated that the EPF, *B. bassiana*, had a great effect on the fecundity of *Eriopis connexa* (Germar). However, there is a few information about interactions with other natural enemies in coccinellid species in general and *C. montrouzieri* in particular. Even though, many coccinellid species and EPF may occupy in the same spatial and temporal habitat.

The present study revealed that EPF species greatly affect the biotic processes as growing stages, fertility, and elements of life tables of *C. montrouzieri*. The instinctive ability for the augmentation (r) and the limited average of augmentation (λ) of *C. montrouzieri* treated with LC_{50} concentration of *B. bassiana*, *M. anisopliae*, and the distilled water (control) were significantly different.

The life table elements of the remedied females are visible in Table 6. The final propagation rates (R_0) in LC_{50} concentration of *B. bassiana* and *M. anisopliae* were significantly lower than the control (155.99, 115.55, and 338.82, respectively). Furthermore, the intrinsic rate of increase (r_m) (0.08, 0.06, and 0.13) and the finite rate of increase (λ) (1.08, 1.06, and 1.14) were less in numerical values than those obtained in the control. Average generation period (T) displayed a clear difference in the LC_{50} groups of both *B. bassiana* and *M. anisopliae* than the control. The longest average generation period was recorded in *M. anisopliae* and *B. bassiana* treatments, but the shortest average generation period was registered in the control (76.78, 60.95, and 43.76). With regard to the productivity of female, it had the highest fertility rate ($\Sigma\ m_x$) (574.9) in the control compared to *B. bassiana* (431.39) and *M. anisopliae* (360.4).

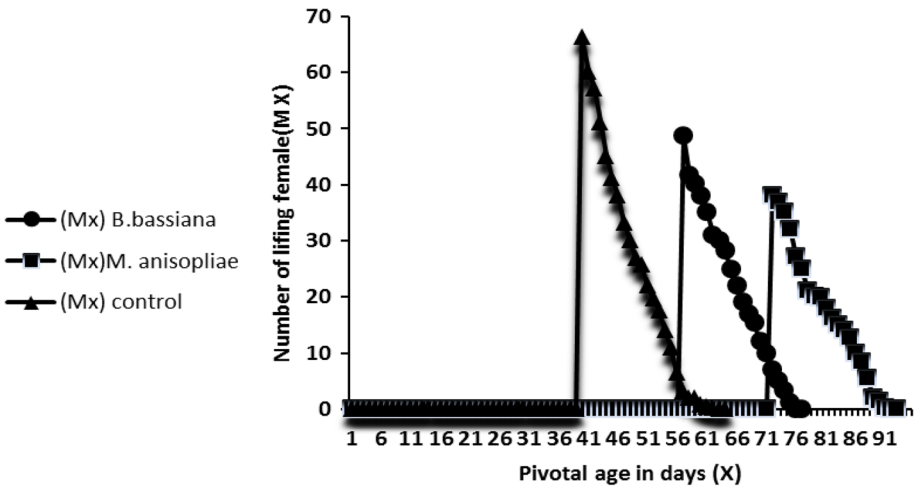

Fig. 2 *Cryptolaemus montrouzieri* female fertility treated with the two tested entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*) and the control

Table 5 LC_{50} effect of entomopathogenic fungi and control on different life periods of *Cryptolaemus* montrouzieri

Fungi isolates	Duration (day, mean ± S.E.)			Total life cycle
	Pre-oviposition period	Oviposition period	Post-oviposition period	
Beauveria bassiana	10.13 ± 0.91	15.14 ± 1.91	5.14 ± 1.03	77.11 ± 1.01
Metarhizium anisopliae	17.13 ± 0.92	19.21 ± 2.51	2.18 ± 1.16	94.08 ± 2.15
Control	6.23 ± 1.70	21.13 ± 1.48	3.22 ± 1.12	64.11 ± 3.15

The outcomes presented in Table 6 displayed that the doubling time (D_T) recorded 8.66, 11.55, and 5.33 in LC_{50} concentration of *B. bassiana* and *M. anisopliae* treatments and the control, respectively. This is on account of the truth that large numbers of larvae and pupae died before they developed into an adult. This proves that the influence of *M. anisopliae* and *B. bassiana* was not only active and destructive in a certain treated stadium, but also had an impact on a subsequent stadium furthermore difficulty in molting (Malarvannan et al., 2010). Little researches have been done on the effect of fungus *B. bassiana* on the reproductive factors in ala decline in the fertility of *Eriopis connexa* predator under laboratory conditions (Ana et al., 2017). The applications of *M. anisopliae* decreased the offspring and affected the duration of survival of *Menochilus sexmaculatus* adults and prevented the growth of larvae to adult (Trizelia et al., 2017).

Even though the laboratory-reared insects are more oversensitive to pathogens (Hajek and Butler, 2000), the ecological elements and behavioral receptions of the predators should be taken into consideration to clarify the rare rate of EPF infesting coccinellids beneath naturalistic field environment.

Conclusion

This study demonstrated that a single concentration of the fungi *B. bassiana* and *M. anisopliae* affected the development, fecundity, and life table elements of the predator *C. montrouzieri*. The concept of the ecological results of utilizing more than one biological control agent required exact testing inside intricate ecosystems, which included different species of natural enemies. Such studies are necessary to assist in describing the interactions between entomopathogens and natural enemies to raise and encourage chances in developing biological pest control programs.

Table 6 Population and reproductive measurements of *Cryptolaemus montrouzieri* females

Parameters	Control	*B. bassiana*	*M. anisopliae*
R_0	338.82	155.99	115.55
T	43.76	60.95	76.78
r_m	0.13	0.08	0.06
λ	1.14	1.08	1.06
D_T	5.33	8.66	11.55
$\sum M_x$	574.90	431.39	360.40

R_o the final propagation rates; $R_o = \sum l_x m_x$, T average generation period; $T = \ln R_o/r_m$; r_m the instinctive ability for the augmentation, $r = \ln R_o/T$; λ the limited average of augmentation, $\lambda = e^r$; D_T twice the amount time, the number of days required by a population to double, $D_T = \ln 2/r_m$; $\sum M_x$ overall reproduction rate

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Author's contributions
I am the only author of this manuscript. So, I am responsible for all the steps. The author read and approved the final manuscript.

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