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Effect of Oleander Aphid (*Aphis nerii* Boyer de Fonscolombe) on the Mortality and Biological Parameters of Green Lacewing (*Chrysoperla carnea* Stephen)

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Authors' Contribution
MS developed basic ideas and was also responsible for experiment and theoretical setting, data collection and analysi and writing of manuscript. RMM conducted experiment and provided guidance during writing of manuscript. QAS helped in developing basic ideas, layout, experiment and theoretical setting and provided guidance for writing of manuscript.

Key words
Oleander aphid, *Aphis nerii*, *Chrysoperla carnea*, Life parameters, Survival analysis.

ABSTRACT

The generalist predator, green lacewing, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) has a key role in integrated pest management (IPM) strategies for many crop pests. The influence of different prey types has been studied to strengthen the *C. carnea*. Current in-*vitro* study was made to figure out the effects of different prey on mortality and certain biological characteristics of *C. carnea.* The highest mortality rate (66.43±2.13%) was for larvae, which fed upon Oleander aphid, *Aphis nerii*. Less larval mortality (7.86±2.37%), larval period (164.57±8.16 h), maximum pupal weight (9.86±0.41 mg) and emergence (95.13±2.03%) was observed when *C. carnea* was provided with eggs of *Sitotroga cerealella*. *Bervicornye brassicae* was performed well than *A. nerii* but was found least successful as compared to eggs of *S. cerealella*. Both aphid species were significantly good in performance when fed to *C. carnea* larvae with *S. cerealella* eggs than their solo effect. Results depicted that mortality factor and life parameters of *C. carnea* larvae are influenced by its prey type which they fed on. These results of the study could be used to improve the rearing and conservation strategies to increase *C. carnea* population and their predatory activities.

INTRODUCTION

There has been a considerable change in adaptation of protocols in insect pest management (IPM) with the advent of concept of organic farming. Natural enemies play a vital role in ecosystem, posing a valid substitute to, or integration with, other control strategies (McEwen *et al*., 2007; Javed *et al*., 2019). So, the development of IPM seeks to upsurge natural control by conservation and preservation of entomophagous fauna (Rogers *et al*., 2007; Rumpf *et al*., 1997). Thus, the impact of different management practices on biocontrol agents; needs to be restudied and improved.

The *C. carnea* is a widespread natural predator of the agroecosystem. It has great potential in IPM of many different crops and non-crop habitats (Duelli, 2001). Generalist predator has played a vital role especially in managing different crop pest (Bompard *et al*., 2013). Due to quick adoptability against adverse climatic factors, high searching capacity and capability of managing a wide range of insect pests make this insect as a successful predator in agroecosystem (Mansoor *et al*., 2013). It is widely spread in whole Holarctic, and preferentially feed on aphid but can also consume soft bodied arthropods like whiteflies, mealy bugs, thrips, scales, leafhoppers and few caterpillars (Khan *et al*., 2010; Syed *et al*., 2005). This predator has been mass-reared and augmented against several insect pests of European and American countries (Balouch *et al*., 2016).

Effective mass rearing protocols need to be established, to produce excessive numbers of predatory lacewings. Quantity and quality of prey species are found influential for biology and behavior of *C. carnea* (Strohmeyer *et al*., 1998; Thompson and Hagen, 1999). Important considerations while evaluating the potential of a predator, are suitability and fitness of prey for the development of predator for deciding its success rate in ecosystem (Hodek, 1993). Although, *C. carnea* is found to be a highly aphidophagous predator, but its biology can be overwhelmed by some aphid species. It is documented that *Aphis fabae* Scopoli was an unsuitable prey for *C. carnea*, as it caused high larval mortality, the formation of smaller cocoons and lower fecundity (Osman and Selman, 1993). However, Nakamura *et al*. (2000) reported that *Chrysopa pallens* cannot develop into an adult on *Tetranychus urticae* as feed.

Oleander aphid, *Aphis nerii* Boyer de Fonscolombe

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(Homoptera: Aphididae) has access to high contents of oleandrin and nerrin in the phloem of plants of Asclepiadaceae and Apocynaceae. These toxic compounds are digested by aphids, sequestered and excreted in honeydew (Malcolm, 1990). Certain prey when consumed by predators, arrest their growth and development, or cause their mortality at an early stage; other are rejected (Panizzi and Parra, 2012). For example, various coccinellids cannot continue their life cycle after consuming *A. nerii* fed oleander plant, since it possesses the toxic active ingredient oleandrin (Iperti, 1966). This allelochemical makes the prey unpalatable and might be the reason for rejection by some predators (Hodek, 1993).

The current study was planned to analyse the influence of two prey species (*A. nerii* and *B. brassicae*) along with *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) eggs on the biology of *C. carnea.*

MATERIALS AND METHODS

The experiments were carried out under controlled laboratory conditions (25±1°C and 60±5% RH) at biological control laboratory, Nuclear Institute of Agriculture (NIA), Tando Jam, during the year of 2017.

S. cerealella *culture*

S. cerealella culture was maintained on wheat grains. For the purpose, wheat grains were pre-treated and disinfected by Phostoxin® tablets (aluminum phosphide) and then poured into jute sack and were sterilized with boiling water for 2 min. The treated grains were then exposed to sun light to eliminate stored grain mites and other potential pests. After solar exposure, half kilogram of grains were then transferred to each 4 liters (20.5 cm L x 55.8 cm D) glass jars. Freshly laid eggs of *S. cerealella* (5ml eggs/jar) were properly mixed with grains to each glass jar. After mixing them properly and were kept in controlled laboratory conditions at (25±1°C and 60±5% RH and 15:7 (L:D) hrs). After hatching, young larvae of *S. cerealella* started feeding on wheat grains till adult emergence. Newly emerge adults were allowed to fed on grains and collected by motorized aspirator and shifted to eggs collecting jars (1L plastic jar with a fine mesh (mesh size = 50) at the bottom). Eggs collecting jars were placed over starch and fresh eggs were collected and further used in experiments.

Rearing of C. carnea

C. carnea eggs were collected from biocontrol lab of Plant Protection Division of Nuclear Institute of Agriculture (NIA), Tando Jam. and placed under cloth cover for hatching. To avoid the cannibalism, individual larvae were shifted in 2 inches polypropylene transparent aerated straw with food (*S. cerealella* eggs) and placed them in controlled biocontrol laboratory at 25±1°C and 60±5% RH and 15:7 (L:D) hrs till pupation. These tubes were cut out from both sides and shifted on glass Petri plates (9 cm diameter and 1.5 cm height) for adult emergence. On emergence, adults were transferred to insect rearing cage (24.5 x 24.5 x 24.5 cm) with black cloth fitted at the top for egg laying substrate. Adults were provided with an artificial diet (honey, sugar, yeast and distilled water (1:2:1:2)) throughout reproductive phase *C. carnea.*

Collection of aphid species

During the studies, *B. brassicae* and *A. nerii* were regularly collected on daily basis from brassica and oleander plants grown at farm area and the residential colony of NIA, Tando Jam, respectively. The collected species were used as prey for *C. carnea*.

Effect of prey on larval mortality of C. carnea

Thirty, 2nd instar larvae of *C. carnea* were collected with fine camel hair brush and individual larvae were shifted into 2 inches polypropylene transparent straw tubes for each treatment. There were five treatments with 30 replication (total 150 larvae were used in the experiment). Five treatments (*A. nerii* (n=20), *B. brassicae* (n=20), eggs of *S. cerealella* (150 mg), *A. nerii* + eggs of *S. cerealella* (n=10+75 mg) and *B. brassicae* + eggs of *S. cerealella* (n=10+75 mg)) were evaluated for *C. carnea* larval mortality. Individual *C. carnea* larvae were fed regularly to avoid starvation. These tubes were placed in an incubator under optimum conditions mentioned above. Mortality was recorded after 12 h interval till pupation.

Effect of prey on life parameters of C. carnea

The above mentioned procedure was recurred to evaluate the effect of different prey on life parameters. Ten, 2nd instar *C. carnea* were used for each treatment (as mentioned above). 4th instar aphids were removed from population to avoid parthenogenesis. Each *C. carnea* larva was monitored daily. The remaining aphids and eggs were removed from tubes and were supplied with fresh aphid and eggs till pupation. Each treatment was evaluated in 5 replicates to determine larval duration, percent larval mortality, pupal duration, pupal weight, percent emergence and sex ratio.

Statistical analysis

Experimental data was statically analysed using SAS JMP® Pro 12.2.0 software to evaluate the significant difference between treatments. Survival analysis was performed to analyse the survivability of *C. carnea* larvae.

Log-Rank and Wilcoxon test were used to calculate the difference in the *C. carnea* larvae survival, between different larval preys.

Furthermore, ANOVA was used to determine the effect of different preys on life parameters like larval duration, percent larval mortality, pupal duration, pupal weight, percent emergence and sex ratio. Tukey's honestly significant difference (HSD) test was used to compare the means of different response parameters.

RESULTS

Survival analysis was used to estimate the expected failure time of *C. carnea* larvae on providing with different larval preys. Each prey showed a significant difference in overlaying steps of estimated survival function. The highest mortality was recorded on providing *C. carnea* provided with *A. nerii*, while minimum mortality was found on *S. cerealella eggs*. When both (*A. nerii* and *S. cerealella* eggs) were provided at the same time, survivability increase and was more than *B. brassicae* (resulting in 2nd highest mortality). In the case of *A. nerii, C. carnea* was started dying just after 36 h, while in the case of *S. cerealella*, first death occured after 60 h of starting up time (Fig. 1).

Accrual time for all treatments was 192 h. It also caution us about their survival even after end of trail time. The corresponding p-value is <0.0001 and thus significant difference was observed in overall survivorship when between larvae provided will different preys (Table II). No significant mortalities were found, when provided with *S. cerealella* eggs alone or with other aphid species. Summary of the survival plots for *C. carnea* larvae when provided different prey type in Table I. Table II gives the Chi-square values and p-values for the prey types when subjected to *C. carnea* larvae.

Significant differences were found in larval duration ($F = 5.28$, $df = 4$, $P = 0.0024$) of *C. carnea* when provided with five different larval preys (Fig. 2). In the case of *A. nerii,* maximum hours (197±3.51) were recorded as a larval period. Minimum larval duration (164.57±8.16 h) was observed in the case of *S. cerealella* eggs. No significant difference was observed in larval period when eggs were provided alone or mix with aphid species.

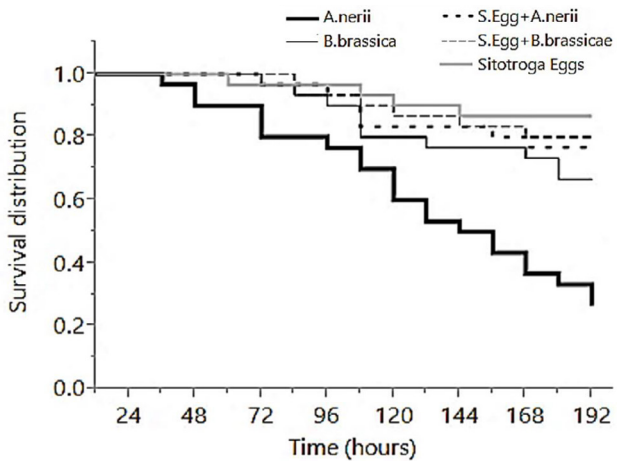

Fig. 1. Survival plot of *C. carnea* larvae reared *in vitro* provided with different larval preys. Preys (*A. nerri*, *B. brassicae*, *A. nerri* + *S. cerealella* eggs, *B. brassicae* + *S. cerealella* eggs and *S. cerealella* eggs) were evaluated for the survival of *C. carnea* larvae. Legends identify different food sources by different line styles. Survival difference between larval preys was detected using a Log Rank and Wilcoxon test.

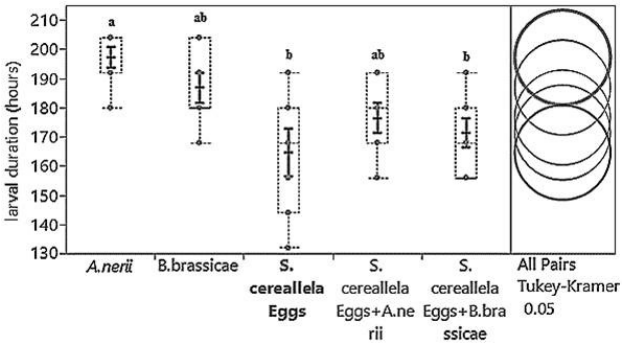

Fig. 2. Larval duration of *C. carnea* is presented in this graph when provided with different larval preys. Black dots are denoting the values of larval duration in hours. The dotted line is presenting box plots and displaying means larval duration in response to various larval preys. All black solid vertical lines in box plots indicate ± SEM. Black circles at right, are presenting the treatment mean comparisons by using Tukey HSD.

Table I.- Summary statistics of survival plot for *C. carnea* larvae reared *in vitro* provided with different food sources.

Group	No. failed	No. censored	Mean	Survival (%)	Std. Error
A. nerri	22	8	139.2	26.7	9.50
B. brassicae	10	20	161.2	66.7	6.61
A. nerri + *S. cerealella* eggs	7	23	156	76.3	5.22
B. brassicae + *S. cerealella* eggs	6	24	158	80	4.84
S. cerealella eggs	4	26	139.2	86.6	3.49

Table II.- Chi-Square approximations for Log-Rank test and Wilcoxon test.

Test	Chi-Square	df	Prob.>Chi-Sq.
Log-Rank	35.682	4	<0.0001*
Wilcoxon	32.566	4	<0.0001*

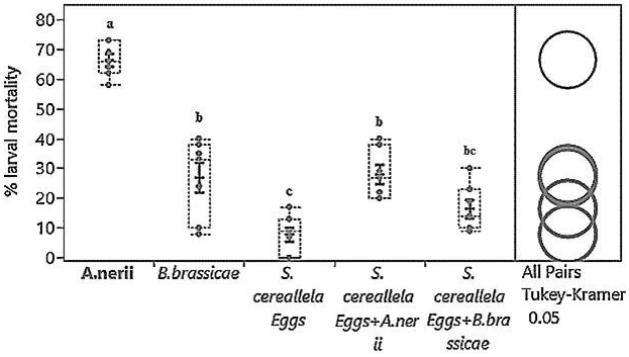

Fig. 3. Percent larval mortality of *C. carnea* is presented in this graph when provided with different larval preys. Black dots are denoting the values of larval mortalities. The dotted line is presenting box plots and displaying means larval mortality in response to various larval preys. All black solid vertical lines in box plots indicate ± SEM. Black circles at right, are presenting the treatment mean comparisons by using Tukey HSD.

Mortality of *C. carnea* was recorded at each prey type subject to them. Significant differences were observed among larval prey types when compared for larval mortality ($F = 46.67$, $df = 4$, $P < 0.0001$). Mortality concern was greater (66.43±2.13%) in *C. carnea* when fed on *A. nerii*, while lesser fatalities (7.86±2.37%) were observed when provided with *S. cerealella* eggs (Fig. 3). Except for *A. nerri*, no significant differences were found in *C. carnea* mortality at all other prey types.

No impact of prey type was found on the pupal duration of *C. carnea* (Fig. 4). The pupal period of *C. carnea* did not differ when fed on different prey types ($F = 2.19$, $df = 4$, $P = 0.094$).

The significant effect was found on the pupal weight when fed five different larval prey types ($F = 11.74$, $df = 4$, $P < 0.0001$). Maximum pupal weight (9.86±0.41 mg) was attained when *C. carnea* was reared on eggs of *S. cerealella*. The combination of eggs of *S. cerealella* with both *A. nerri* and *B. brassicae* produced more healthy pupae and attained weight 7.01±1.02 mg and 8.43±0.53 mg, respectively. Minimum pupal weight (4.42±0.43 mg) were recorded, when *C. carnea* reared on *A. nerri* solely (Fig. 5).

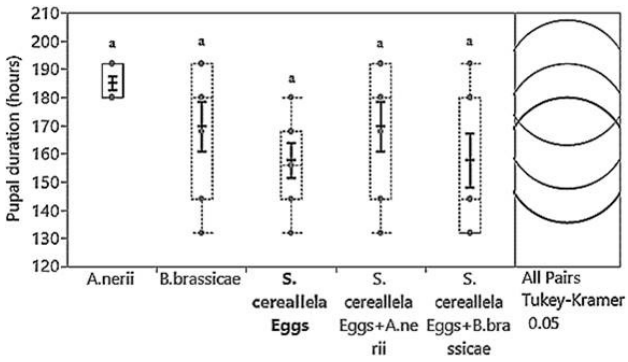

Fig. 4. Pupal duration of *C. carnea* is presented in this graph when provided with different larval preys. Black dots are denoting the values of pupal duration in hours. The dotted line is presenting box plots and displaying means pupal duration in response to various larval preys. All black solid vertical lines in box plots indicate ± SEM. Black circles at right, are presenting the treatment mean comparisons by using Tukey HSD.

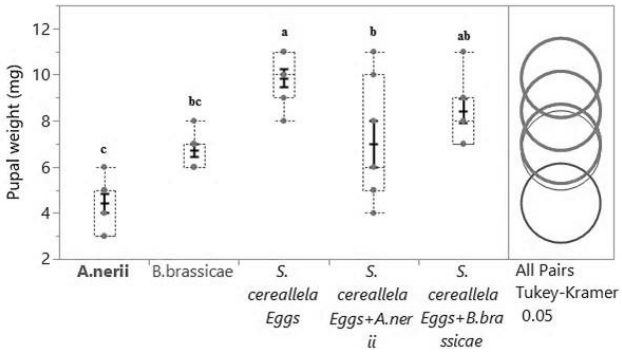

Fig. 5. The pupal weight of *C. carnea* is presented in this graph when provided with different larval preys. Black dots are denoting the values of pupal weight in milligrams. The dotted line is presenting box plots and displaying means pupal weight in response to various larval preys. All black solid vertical lines in box plots indicate ± SEM. Black circles at right, are presenting the treatment mean comparisons by using Tukey HSD.

Percent adult emergence of *C. carnea* feeding on the five prey types is shown in Figure 6. Significant differences were found in percent adult emergence ($F = 63.98$, $df = 4$, $P < 0.0001$). the mean comparison showed that *C. carnea* larvae fed by *S. cerealella* eggs alone (95.13±2.03%) and combined with aphid species (*A. nerii* = 73.57±2.67 %; *B.brassicae* = 84.4±2.50%) had significant more adult emergence than fed alone on aphid species. Minimum adult percentage (33.77±2.9) was recorded when C. carnea fed by *A. nerri* alone.

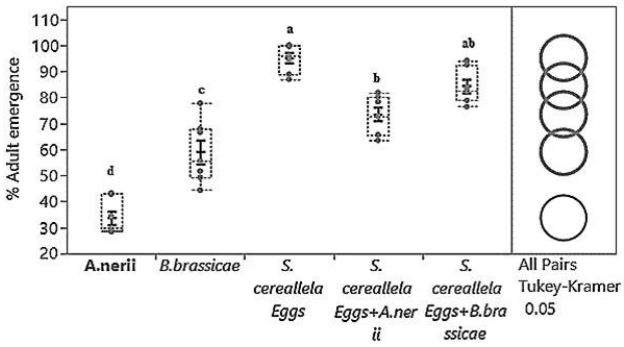

Fig. 6. Percent adult emergence of *C. carnea* is presented in this graph when provided with different larval preys. Black dots are denoting the values of adult emergence. Dotted line is presenting box plots and displaying means adult emergence in response to various larval preys. All black solid vertical lines in box plots indicate ± SEM. Black circles at right, are presenting the treatment mean comparisons by using Tukey HSD.

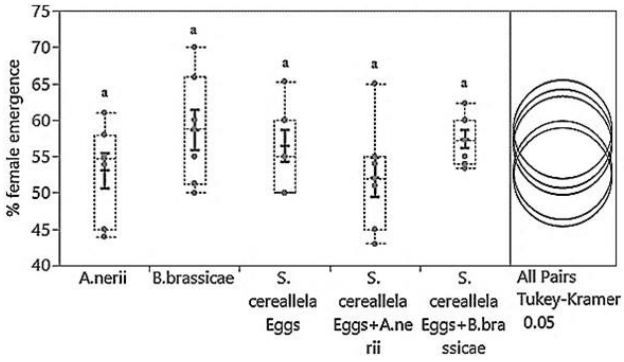

Fig. 7. Percent female emergence of *C. carnea* is presented in this graph when provided with different larval preys. Black dots are denoting the values of female emergence. The dotted line is presenting box plots and displaying means female emergence in response to various larval preys. All black solid vertical lines in box plots indicate ± SEM. Black circles at right, are presenting the treatment mean comparisons by using Tukey HSD.

Percent female emergence out of the total adult emergence of *C. carnea* feeding on the five prey types is shown in Figure 7. There was no significant different ($F = 55.94$, $df = 4$, $P = 0.2361$) found after analysis of variance.

DISCUSSION

Although *C. carnea* is most voracious predator of many species of aphids and is well known as the "aphid lion", but quality and quantity of larval food is considered important and plays a vital role in successful completion of their growth and development and subsequent adult performance (Nandan *et al*., 2014). To assess the impact of different larval prey types on mortality and life parameters of *C. carnea*, a laboratory experiment was conducted. A significant difference was observed in mortality of *C. carnea* larvae when fed to the various prey types. The difference in the *C. carnea* larvae survival, between different larval preys was detected using both Log-Rank and Wilcoxon test. Because Log-Rank test gives more weightage to large survival times and is more effective when the ratio of different function in groups being compared is almost constant. It is also called as a force of mortality or mortality rate. While, Wilcoxon test gives more weightage to early survival times and is only the suitable rank test when the error distribution is logistic (Kalbfleisch and Prentice, 1980).

During the studies it was observed that most of the *C. carnea* larvae were unable to complete their lifecycle when fed on *A. nerri* alone. Whereas some of them when reached at pupal stage were unable to convert into healthy adults due to lack of appropriate size and weight.

Prior to research, it was supposed that *A. nerii*, possibly would not accomplish the nutritional requirement, and thus growth and development of *C. carnea* larvae will be hampered (Gupta *et al*., 2006). Canard *et al*. (1984) found that its biology and behavior is highly dependent on the food quality which they fed on. Several studies have been reported that *A. nerii* to be an unsuitable food for its predator and parasitoid (Snyder *et al*., 2000; Srivastava, 2003). The finding of our studies depicted that by feeding on eggs of *S. cerealella*, individuals passed through the larval and pupal stage and emerged into adults successfully. While *A. nerii* was recorded to be a poor food for *C. carnea*.

C. carnea growth on *A. nerii* was poor, regardless of chemical or physical variation among host plant species. When fed eggs of *S. cerealella* with either of aphid species, virtually all larvae performed better. Successfully developed pupae that had fed only *S. cerealella* eggs as larvae were approximately double the size that has been fed by *A. nerii*. Even, larval period was recorded significantly prolonged when provided with *A. nerii* than other prey types. These findings coincide with the results that some aphid species used as food not only affect the size of their natural enemies but resulted in slower developmental times as compared to larvae fed on near-ideal diet (Snyder *et al*., 2000).

The ineptness of *A. nerii* as a prey item was previously reported to affect the performance of predators that feed on them as host plant cardenolides sequestered by *A. nerii* (Hodek, 1993). Confiscation of host plant toxins in aphids is well explained in research articles, but less is known

regarding the way in which herbivores and natural enemies process these toxins (Pasteels, 2007). Some aphid species can be toxic due to the high concentration of nerrin and oleandrin, which digested and then sequestered by aphid and excreted in honeydew (Malcolm, 1990).

CONCLUSION

The high amount of cardenolides, cardiac glycosides, particularly nerrin and oleandrin, were identified to be responsible for the toxicity of *A. nerii* attained form host plant, *Nerium oleander* (Rothschild, 1961).Certain prey consumed by a predator doesn't allow healthy development and perform well. Furthermore, poor nutritive value or toxicity of prey leads in, for example, slow development, decreased fecundity or fertility, low weight or ultimately high total mortality (Hodek, 1993).

More research in the field of diet specificity of aphidophagous predator is necessary. Such studies are needed not only for enhancing our information but mainly for rational integrated pest management, specifically about introduction and augmentation. It is also essential for establishing the rearing protocols of *C. carnea* regards their artificial food as well. Prey unsuitability may be a cause why some released predators fail to establish or reared *in-vitro*.

Statement of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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