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Effect of Intra-Guild Predation and Sub Lethal Concentrations of Insecticides on the Predation of Coccinellids

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ABSTRACT

Coccinellids beetles are considered one of the potential predators of the canola and mustard aphids. But the application of insecticides in field to manage aphids cause impairment of several key biological traits of the exposed coccinellids through physiological and behavioral effects. It is crucial to understand the effects of insecticides on the coccinellids. The current research was aimed at determining the intra-guild predation (IGP) of different life stages combinations of *Coccinella septempunctata* and *Coccinella transversalis* in the presence and absence of prey and the impact of sublethal concentrations of insecticides on intra-guild predation (IGP) and aphid consumption by coccinellids. Data revealed that in the absence of prey larvae of *C. septempunctata* exhibited more predation on *C. transversalis* while the predation of *C. septempunctata* on *C. transversalis* was relatively less in the presence of prey. The larvae of *C. septempunctata* were found stronger and competitive than larvae and adults of *C. transversalis* during IGP. Higher IGP of *C. septempunctata* on *C. transversalis* was linked to its larger body size. Moreover, sublethal concentrations of insecticides (lambda-cyhalothrin, cypermethrin, thiamethoxam, imidacloprid, profenophos and chlorpyriphos) significantly affected the IGP of coccinellids beetles and their aphid consumption. The effect of insecticides was concentration dependent. None of the tested insecticides was found completely harmless to coccinellids. Imidacloprid was found relatively safe to coccinellids and hence, recommended for use in canola field. It is concluded that *C. septempunctata* is a stronger predator showing high value of IGP and the presence of prey affects the predation of *C. septempunctata* on *C. transversalis*. Sublethal concentrations of all test insecticides exert a negative impact on IGP and aphid consumption by both these coccinellids species. Imidacloprid is relatively safe for aphid control.

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Authors' Contribution
MAR conceived and designed the experimental protocols. RA performed the experiment. RA and MAR prepared the manuscript and analyzed the data. MA and MZM provided assistance in experimentation and manuscript preparation

Key words
Insecticides, Sublethal doses, Coccinellids, Intraguild predation, Aphids, *Coccinella septempunctata, C. transversalis.*

INTRODUCTION

Beneficial arthropods play a vital role in regulating many insect pest species in agro-ecosystem. Coccinellidae family (Order: Coleoptera) is one of the main group. Their larvae and adults are known to feed voraciously on phytophagous arthropod pests including aphids (Weber and Lundgren, 2009; Ahmed *et al*., 2017; Arshad *et al*., 2018).

Although pesticides play a major role in integrated pest management (IPM) in different cropping pattern, but they are toxic to many predators and parasitoids and the evaluation of pesticide safety to biocontrol agents is usually assessed on acute toxicity basis. Recently, many scientists have paid attention on the sublethal effects of pesticides on natural enemies of insects including their adverse impact on fecundity, behavior, development rate and longevity (Croft, 1990; Desneux *et al*., 2004, 2007; Biondi *et al*., 2012; Delpuech *et al*., 2012; Pekar, 2012; Martinou *et al*., 2013).

Coccinellids are highly vulnerable to chemical insecticides which are sprayed on crops to control sucking insect pests. Generally, the 1st and 2nd instar of ladybird beetles are very sensitive to insecticides in field while these chemicals are very effective against aphids (Youn *et al*., 2003). Some of the insecticides kill all life stages of coccinellids that feed on the treated aphids. Theiling and Croft (1988) showed that coccinellids susceptibility to pesticides differ with the species and type of insecticide and determined that the sensitivity of coccinellid beetles to pesticides was somewhat lesser than that of pests. Insecticides usually affect the physiology and behavior of coccinellids directly (Galvan *et al*., 2005; Desneux *et al*., 2007) and reduced the prey consumption of predators (Provost *et al*., 2005).

In addition to above mentioned effects, the Coccinellids can, under certain conditions due to application of insecticides, engage in competitive interactions (intraguild predation) between members of the same trophic level (Provost *et al*., 2003; Rondoni *et al*., 2014). Interactions among predators are common and may generate complex effects on ecosystem dynamics. Intraguild predation is an interspecific interaction between

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predators sharing a common resource and the outcome is the death and consumption of one of the predators. When intraguild predation is observed in an ecosystem, the population dynamics may be modified in different ways, which in turn may cause environmental instability (Holt and Polis, 1997). Intraguild predation can affect both predator and prey populations, and consequently disrupt the biological control programs (Rosenheim *et al*., 1995).

Most of the previous studies have been focused on direct toxicity and mortality of coccinellids due to insecticides. However, the effect of sublethal doses of insecticides on intraguild predation has not been studied in detail. In current study, firstly we evaluated the intraguild predation (IGP) in the presence and absence of extraguild prey (aphids) among *C. septempunctata* and *C. transversalis*. Secondly, the effect of sublethal concentrations of insecticides on IGP of two coccinellids which are common predator of aphids on canola crop in Pakistan was studied under the laboratory conditions.

Table I.- Sublethal concentrations of insecticides against *C. septempunctata* and *C. transversalis.*

Names of insecticides	*C. septempunctata*		*C. transversalis*	
	$LC_{10\,(ppm)}$	$LC_{30(ppm)}$	$LC_{10\,(ppm)}$	$LC_{30\,(ppm)}$
Imidacloprid	88.7	194.5	17.4	158.7
Thiamethoxam	41.4	135.3	10.8	117.5
Profenophos	3.8	54.3	4.8	56.4
Chlorpyriphos	4.0	70.01	7.9	75.5
Lambda-cyhalothrin	11.7	101.6	13.4	95.9
Cypermethrin	67.1	271.9	29.1	210.4

MATERIALS AND METHODS

Coccinellids (*C. septempunctata* and *C. transversalis*) were collected from canola fields which have never been exposed to insecticides, and reared in insect cages under appropriate temperature and relative humidity (26 ± 1°C, 65 ± 5% R.H) to get the homogeneous stages of above mentioned predators. Collected adults and larvae were fed on natural diet *i.e.* aphids. Aphid infested twigs were offered to adult and larval predators and provided with water soaked cotton.

Field survey was conducted in brassica growing region and one hundred farmers were questioned about the insecticides sprayed for the control of aphids. Survey results demonstrated six insecticides such as imidacloprid, thiamethoxam, cypermethrin, lambda-cyhalothrin, profenophos and chlorpyriphos, were sprayed against brassica aphids, hence, used in our study. LC_{10} and LC_{30} of above mentioned six formulated insecticides to *C. septempunctata* and *C. transversalis* was determined by making four serial dilutions of insecticides resulting 10 to 90% mortality. Adult beetles were treated with four concentrations of each insecticide using topical method of application (Paramasivam and Selvi, 2017). One microlitre of each concentration was applied on the dorsal side of abdomen of beetle using micropipette. Adults were kept on ice for 5min to prevent their movement before application. A total of 20 insects with three replications were released in the petri dishes to check the toxicity of each insecticide. Sufficient aphids were added in each petri dish to avoid death of beetle due to starvation. The data of mortality was recorded after 24h of treatments. LC_{10} and LC_{30} were calculated for each insecticide using Probit analysis by running Polo Plus® software (Finney, 1971).

Characterization of intraguild predation in two coccinellid beetles

Evaluation of intraguild prey was done in the presence and absence of extraguild prey (aphids). Experiments were conducted on small canola leaves which were placed on a thin layer of agar in a petri-dish to avoid leaf desiccation. Predators were starved for 24 h before test. The level of intraguild predation was determined for two predator (*C. septempunctata* and *C. transversalis*) combinations. Only mobile stages of each species for combinations of one individual were selected. The combination of egg and pupal life stages were excluded. The following combinations or treatments were selected for IGP with and without prey (Table II).

Table II.- Combinations or treatments selected for IGP with and without prey.

Treatment	*C. septempunctata*	*C. transversalis*
T1	Larvae	larvae
T2	Larvae	Adult
T3	Adult	Larvae
T4	Adult	Adult

An individual of each predator species of the selected combination was introduced on separated leaves. In the presence of extraguild prey, twenty aphids were released in petri dish followed by sealing with transparent plastic film. After 4 h, predator and prey mortality was assessed. The control treatment was consisted of only aphids to evaluate the potential of escape and the natural mortality of the aphids was also included. Fifteen replicates were set for each treatment. The corrected mortality, attributed to predator interaction, was calculated according to Soares (2002).

$$P\,(IGP) = (t - a) \times r\,a$$

Where; *P* is the number of replicates with predation (intraguild predation), *t* is total number of replicates, *a* is number of replicates where the individual was alive and *ra* is the ratio of alive individuals in the control.

An index of symmetry (SI: corrected mortality in which a given predator was eaten over the total number of replicates in which there was intraguild predation) was calculated for each pair of predators and it was compared with a theoretical index of 50% (corresponding to a symmetric interaction) using a test of conformity. For each predatory pair, the level of intraguild predation (IGP: corrected mortality over the total number of replicates) was calculated.

Table III.- Combinations or treatments selected for the evaluation of predation efficacy of coccnellids.

Treatments/ combination	*C. septempunctata*	*C. transversalis*
T1	Adult	-
T2	Larvae	-
T3	-	Adult
T4	-	Larvae
T5	Larvae	Larvae
T6	Adult	Adult
T7	Larvae	Adult
T8	Adult	Larvae

Impact of sublethal concentration of insecticides on intraguild predation and predation efficacy

The predation efficacy, corresponding to the number of prey consumed and mortality of coccinellid larvae and aphids was evaluated for both species of coccinellids separately and in combination to evaluate the impact of IGP. To this end, small canola leaves were treated with two sublethal concentrations (LC_{10} and LC_{30}) of insecticides and were placed on a thin layer of agar in a petri-dish to avoid leaf desiccation. Predators were starved for 24 h before test. Predation efficacy was evaluated for all treatments/combinations (Table III) with a method as mentioned earlier.

There were five replicates for each treatment along with control treatment in which potential of escape and natural mortality was evaluated.

RESULTS AND DISCUSSION

Sublethal concentrations of insecticides

Results of bioassays of *C. septempunctata* exhibited that the sublethal concentration LC_{10} of profenophos, chlorpyriphos, imidacloprid, thiamethoxam, lambda cyhalothrin and cypermethrin was 3.8, 4, 88, 41, 11 and 67µL/L, respectively and LC_{30} of all earlier mentioned insecticides was 54, 70, 194, 135, 101 and 271 µL/L, respectively. The bioassays of *C. transversalis* against insecticides showed that the sublethal concentration LC_{10} of imidacloprid, thiamethoxam, profenophos, chlorpyriphos, lambda cyhalothrin and cypermethrin was 17, 10, 4.8, 7.9, 13 and 29µL/L, respectively and LC_{30} of above mentioned insecticides was 158, 117, 56, 75, 95 and 210µL/L, respectively (Table I). It is likely that both species of coccinella showed similar response to chemical insecticides. Overall, on the basis of lethal concentration, it was concluded that organophosphates revealed high toxicity to *C. septempunctata* and *C. transversalis* and compared to neonicotinoids and pyrethroids.

Among the insecticides, cypermethrin and imidacloprid exhibited lower toxicity to *C. septempunctata* and *C. transversalis*, hence considered relatively safe.

Intraguild predation in the absence/presence of prey

In the absence and presence of prey (aphids), the intraguild predation between *C. septempunctata* and *C. transversalis* was observed (Fig. 1). Intraguild predation can happen between aphidophagous predators therefore dropping their efficiency in regulating the crop pests. Between ladybirds, *C. septempunctata* L. and *C. transversalis* are the best operative predators upon aphids, the economically significant pest of canola (Rondini *et al*., 2014). In the absence of extraguild preys, there was more intraguild predation in most of the combination of life stages of both predators compared to presence of extraguild preys (Lucas *et al*., 1998). Previously, intraguild predation has also been observed in *C. septempunctata* and other coccinellids (Yang *et al*., 2017), however, IGP between *C. septempunctata* and *C. transversalis* is not well studied.

In the absence of prey (aphids) (Fig. 1A), the larvae of *C. septempunctata* showed more predation on *C. transversalis* larvae and adults as compared with the presence of prey (Fig. 1B). The adult of *C. septempunctata* have same level of predation on *C. transversalis* larvae and adults in the presence and absence of prey. The larvae and adult of *C. transversalis* also exhibited very low predation on larvae and adult of *C. septempunctata* respectively. It is likely that the larvae of *C. septempunctata* are good survivors in the presence and absence of prey. In the absence of prey, they start feeding on other coccinellids species or alternative to their prey for their survival which is a very useful characteristic of a good predator. Overall, *C. septempunctata* was superior to *C. transversalis*. This might be due to size of larvae and adults.

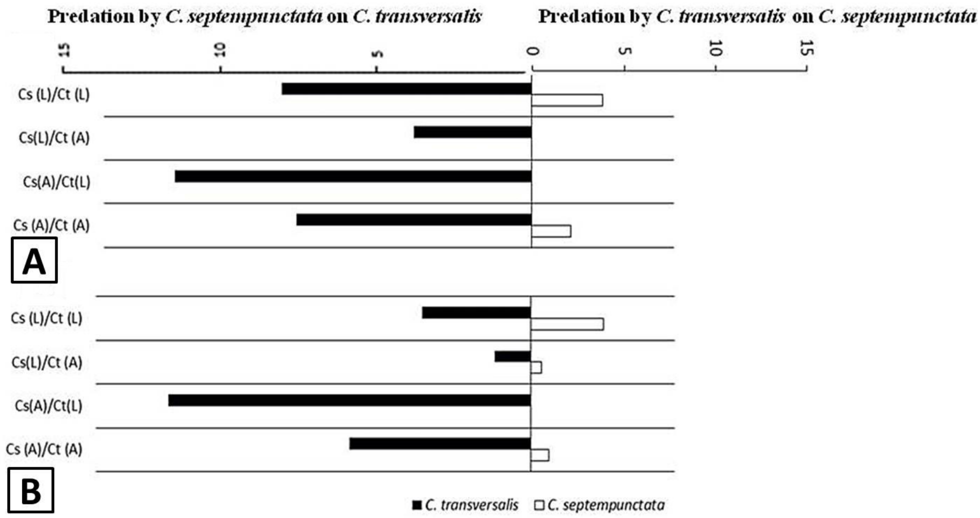

Fig. 1. Levels of intraguild predation (IGP) of different life stage combinations of *C. septempunctata* and *C. transversalis* in the absence of extraguild prey (A) and in the presence of extraguild prey (B). X-axis shows the consumption of one predator species by the other predator species. Open bars shows intraguild predation of *C. transversalis* on C. *septempunctata* and shaded bars shows intraguild predation of *C. septempunctata* on *C. transversalis*. Cs (L), *C. septempunctata* larva; Cs (A), *C. septempunctata* adult; Ct (L), *C. transversalis* larva; Ct (A), *C. transversalis* adult.

C. septempunctata were bigger than *C. transversalis*. Our results are in agreement with other scientists (Sengonca and Frings, 1985; Rosenheim *et al*., 1995; Cottrell and Yeargan, 1998; Lucas *et al*., 1998; Phoofolo and Obrycki, 1998; Felix and Soares, 2004).

Impact of insecticides on the intraguild predation and predation efficacy

Predation efficacy of *C. septempunctata* and *C. transversalis* larval and adult life stage alone and in combination was significantly affected by sublethal concentrations of all insecticides (Fig. 2A-F). In the absence of insecticides, the predation efficacy was significantly higher in control of all treatments. Larvae and adults of *C. septempunctata* exhibited higher predation efficacy as compared to *C. transversalis*. In all treatments, the predation efficacy was concentration dependent. The LC_{30} of all insecticides exhibited significantly less predation efficacy as compared to LC_{10} and control. Overall, the combination of larval and adult life stages of *C. septempunctata* and *C. transversalis* (T7 and T8) exhibited significantly higher predation efficacy as compared to life stages alone. But these treatments were not significantly different from each other under different insecticidal concentrations (Fig. 2A-F).

Predation efficacy of *C. septempunctata* and *C. transversalis* in different combinations/treatments was significantly affected by insecticides applied at two sublethal concentrations (LC_{10} and LC_{30}). Application of all insecticides (imidacloprid, thiamethoxam, profenophos, chlorpyriphos, lambda-cyhalothrin and cypermethrin) significantly decreased the aphid consumption compared to untreated/control treatment (Fig. 2A-F). Among sublethal concentrations of all insecticides, significantly more aphids were consumed which were treated with LC_{10} compared to $LC_{30.}$ None of the combinations was considered best for predation. Overall, aphid consumption was high in combinations (from T5 to T8) as compared to the larvae and adults alone (from T1 to T4) (Fig. 2A-F). Profenophos, chlorpyriphos and cypermethrin significantly reduced the aphid consumption to 50% of coccinellids in all treatments (Fig. 2C, D, F), while imidacloprid, thiamethoxam and lambda-cyhalothrin (Fig. 2A, B, E) exhibited relatively higher aphid consumption but remained significantly lower than control. Previous studies showed that application of insecticides may cause knockdown or repellent effect on predators due to which preying efficiency can be reduced (Croft, 1990). Application of insecticides can also hyper-activate the predator as reported in *Coleomegilla maculata* after application of malathion which caused reduction in prey consumption (Roger *et al*., 1995). There may be following three reasons which caused reduction in the consumption of aphids by coccinellids beetles. First, the insecticides having repellent properties such as lambda-cyhalothrin may also increase the mobility of predator which do not feed on preys and search for untreated area (Croft, 1990). Second, after application of insecticides, the prey become

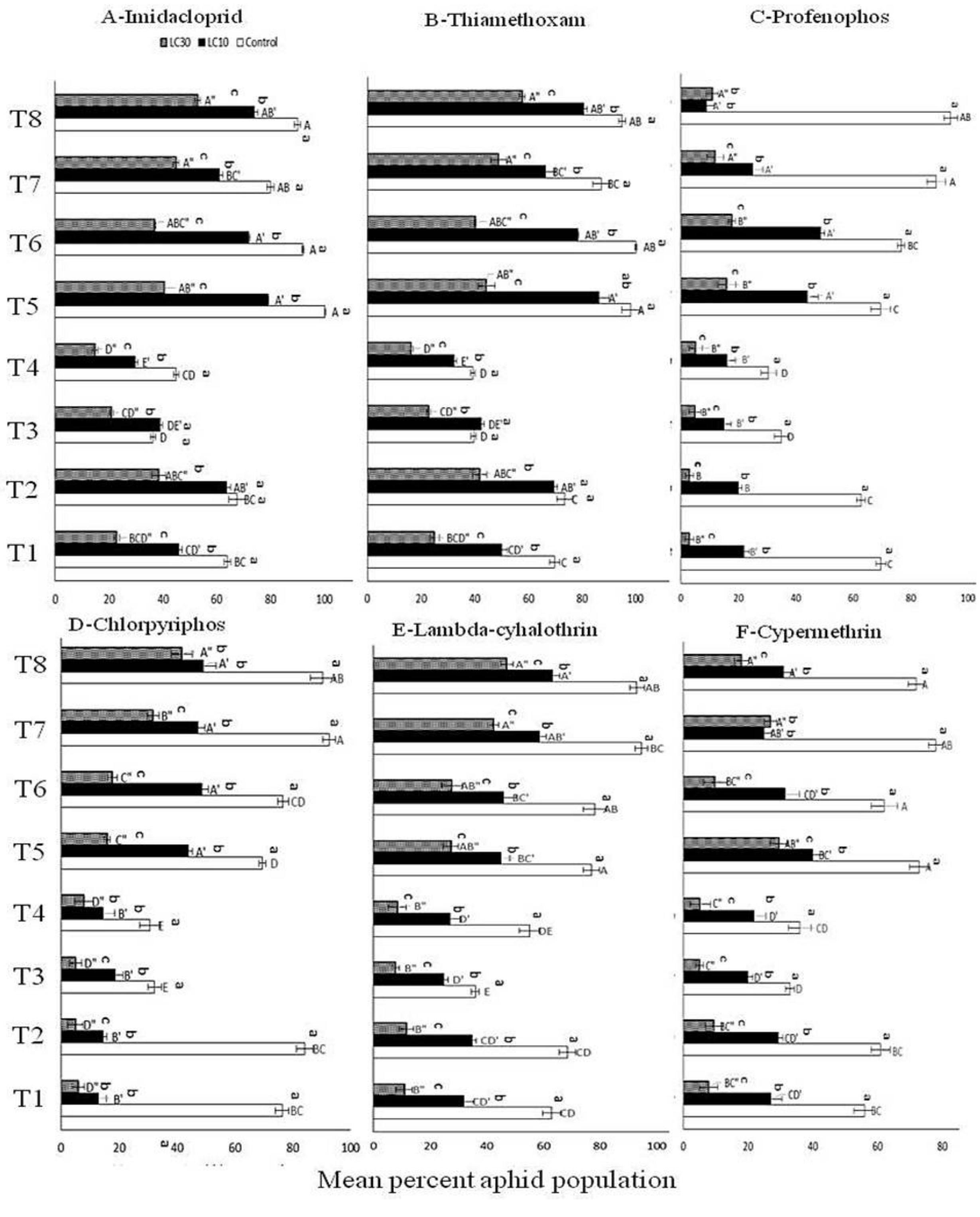

Fig. 2: Predation efficacy of larvae and adult of *C. septempunctata* and *C. transversalis* in combination and alone in the presence and absence of insecticides. Upper case letters shows the comparison of all controls in all treatments; Upper case letters with apostrophe (') shows comparison of LC_{10} of all treatments; Upper case letters with inverted commas (") shows comparison of LC_{30} of all treatments; Lower case letters shows the comparison among control, LC_{10} and LC_{30} of each treatment.

more mobile than predator that in turn must be more efficient to attack preys. Third, insecticide treated preys may be rejected by the beetles (Provost *et al*., 2003). Our results are in agreement with Provost *et al*. (2003, 2005). Although, these laboratory studies are more accurate and specific compared to semi-field and field conditions, but these studies lack the complexity of natural ecosystem and underestimate the effect of pesticides on coccinellids. Therefore further studies are required under field conditions in order to better comprehend the impact of sublethal concentrations of insecticides on intraguild predation and predatory efficacy of predatory coccinellids.

CONCLUSION

It is concluded that none of the tested insecticides was completely safe for coccinellids, however, the neonicotinoid, imidacloprid appeared relatively safer because it did not affect the aphid consumption by coccinellids and hence is recommended for aphid control in canola crop.

Statement of conflict of interest

The authors declare that there is no conflict of interests regarding the submission and publication of this work.

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