Assessing Reptile Cognition and its Role in Biological Pest Control Amidst Future Climate Warming in a Tropical Agroforest Landscape in India

34363-B- 1st Booster Grant

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Summary

Agriculture faces risk from an increase in pest outbreaks this is particularly problematic in developing nations like India that are heavily dependent on food cultivation. Biological pest regulation is a sustainable strategy to mitigate crop pests and a pertinent solution considering the negative impacts of pesticides on the environment, biodiversity, crop quality, and human health. However, little is known about factors that impact the behaviour and efficiency of the pest predators and so the effects of biological pest regulation can be unpredictable. Current approaches to biological pest regulation assess the importance of a pest controller by examining its ability to maintain pest populations over an extended period such that they oscillate between their upper and lower limits. However, this approach lacks efficiency, specificity, and efficacy because it does not take into account crucial factors which determine how predators find, evaluate and remember food sources - the cognitive processes underlying their behaviour.

This project investigated the cognitive factors involved in biological pest control and examined how these factors impact pest controller performance. In this context, reptiles are an ideal group of natural biocontrol agents. Their pest control ability expands across a wide taxonomic range of arthropod orders including Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Odonata, Araneae, and Orthoptera. This broad coverage requires an array of cognitive challenges; the animals must use multi-sensory cues to detect and select prey as well as retain information about prey position, quality, and quantity. There is evidence of remarkable cognitive abilities in this group. Using the Oriental garden lizard (*Calotes versicolor*) as a model species, this interdisciplinary project attempted to augment biological pest regulation strategies by investigating the role that cognition plays in predator performance. Reptiles proved to be efficient in learning the position of highly infested crop patches, making correct choices of selecting the better of the two patches (in terms of pest availability) following colour and position cues. Reptiles showed evidence of colour and taste discrimination abilities. The fundamental knowledge generated from this project is crucial to improve our approach towards bioregulation. This novel project combined the fields of cognitive biology and ecology to improve the success of a key ecosystem service. The goal was to re-examine biological pest regulation through the

lens of animal cognition. The project focused on distinct cognitive abilities – cue learning, position vs colour, taste, quantity and colour discrimination likely to be relevant to service provisioning. It investigated these factors under semi-natural field conditions testing how they impact pest control. Our findings from this project can be expected to pave the way for more implication-based studies in cognition biology aimed at finding solutions to practical challenges like pest mitigation, conservation of species, and services.

State-of-the-art

The United Nations Food and Agriculture Organisation (FAO) estimates a 40% loss in global crops due to insect pests, with the expected loss of attainable yields being 65–80% (Oerke & Dehne, 2004). This has significant financial implications, accounting for a loss of more than $ 470 billion per year (Culliney, 2014). This crisis comes at a time when our understanding of the effect of agrochemicals on ecosystems, human health, crop quality, crop health, and ecosystem health has resulted in a need to use alternative approaches to manage crop pests. Biological pest suppression is a key ecosystem service that aims at increasing crop yields while minimizing the consequences of using agrochemicals (Ghosh et al., 2023).

Biological pest control uses communities of natural predators with diverse attack rates, feeding capacity, prey range, temporal and spatial occurrence to help control pests. Reptiles play an integral part in this trophic interaction (Fraga-Ramírez et al., 2017). They are efficient bioregulators (Lisiecki, 2019; Monagan et al., 2017) and are generalist and opportunistic predators.

Crop pests exhibit a diverse array of salient features that offer a range of stimuli for predators to learn about (Ghosh et al., 2023). They exhibit an extensive range of color morphs, crypticism, body size, movement patterns, olfactory, auditory, and vibrational cues. In addition, they have diverse spatial and temporal occurrence and infestation types. Their infestation modes are numerous and vary with their life-history phases, for example, some are damaging at their larval stage while others affect the crop in the adult stage. As such, it is important to understand what cues animals use to find, select and remember prey.

Reptiles possess a wide suite of cognitive skill particularly in areas that are likely to be linked to success as a biological pest controller. There is evidence of discrimination using visual (Miletto Petrazzini et al., 2018), olfactory (Lomáscolo & Schaefer, 2010) and auditory cues in this group. They are able to learn to navigate to a goal using different types of information (Wilkinson et al., 2009; Mueller-Paul et al., 2014). The Oriental Garden Lizard (*Calotes versicolor*; Daudin, 1802) is an agamid lizard that is predominantly an insectivore. They are an important part of agricultural biodiversity, they are sit-and-wait predators and exclusively encounter and consume highly mobile prey (Vanhooydonck et al., 2015). Garden lizards are known to rely on both visual and chemical cues from conspecifics during breeding (Ammanna et al., 2014). They have been reported to possess taste buds that allow for taste discrimination between prey and are capable of discriminating between colors and associating colors with specific outcomes (Shanbhag et al., 2010). The ability to learn about the location, temporal availability and relative quality of potential food sources is clearly advantageous for the animal, and evidence suggests that animals can learn about relevant phenomena in laboratory settings. However, the implications of this for successful biological pest control have not been experimentally assessed. Reptiles are considered to be essential biological pest controllers (Monagan et al., 2017). For this proposal, the Solanaceae (eg, brinjal) family of crops were selected for its year-round availability and crickets *Teleogryllus mitratus* was selected as crop pest which are defoliators and pose severe threat to crop plants.

Aim

Theoverarching aim of this project was to assess the aspects of cognition involved in biological pest control and, subsequently, to investigate the role that cognition plays in pest control success.

Objectives:

1. To experimentally assess if garden lizards can distinguish between a high and low-infested crop patch and how that impacts pest regulation

2. To experimentally assess the information that the garden lizards use to discriminate between food patches (position vs colour)

3. To investigate if reptiles can discriminate between taste and colour

General methodology for objectives 1 and 2

Nine Oriental garden lizards participated in this project. The animals were maintained at the School of Ecology and Conservation Laboratory, University of Agricultural Science, Gandhi Krishi Vigyana Kendra (GKVK) under climate controlled environment. Mean temperature of the room was maintained within 24 to 25.9 °C and humidity varied between 57 and 70%. Reptiles were collected from the wild (semi-urban habitat) approximately six kilometers from the study site. Reptiles were housed individually in enclosures. They had free access to water and were fed on a variety of insects including mealworms, crickets and grasshoppers. Reptiles were assessed by a vet twice during the study period (reports attached in the appendix section). This study has received the institutional animal ethics clearance from both the host institution (ATREE) and University of Lincoln along with the Institutional Review Board clearance certificate from ATREE.

Apparatus and setup

Subjects were tested in a semi-natural field set-up that was established within a patch of natural habitat in the Gandhi Krishi Vigyana Kendra (GKVK) campus (Figures below). The experimental arena measured 165 x 126 x 118.5 cm. The walls were made of polymer sheets with the bottom part being dug into the soil. The front of the enclosure was covered with a net to allow access to researchers inside the arena for plot maintenance and releasing and collecting animals. The arena contained two naturalistic foraging patches which were supplemented with varying prey densities. The foraging patches contained crop plants of *Solanum sp.* that represented a typical natural agricultural habitat for this model reptile species. Crops were outsourced from a nursery and 60 brinjal plants at sampling stage were procured and maintained throughout the experimental tenure. Upon completion of the project they were donated to the host institute. Crickets i,e crop pests were outsourced from ATREE. 40-50 days old crckets were procured and maintained on diverse diets like cabbage, pumpkin, brinjal, spinach, ground rice and pulses. This size class of pests made detection easy by lizards easy, as well as us while handling them for releasing and retrieving from the patch.

Procedure

Each reptile was habituated to the set-up in three successive trials. Each habituation trial lasted for 15 minutes in which the lizards were free to explore the plot. This allowed enough time to the lizards to get used to human presence while performing which otherwise might have impacted the results. Habituation trials served four major purposes- a. reduce neophobia, b. reduce performance deters in presence of humans, c. maintained motivation, d. retained their natural forgaging activities and instincts.

Experimental conditions

Each reptile participated in 11 experimental trials. Trials were conducted between 9.00 hr to 13.00 hr which is their peak activity period. Prey availability between two foraging patches was manipulated by adding 15 prey items (crickets, *Teleogryllus mitratus*) to one patch (Enhanced patch-E) while the other patch, non-enhanced patch (NE), did not have any additional prey. The position of the enhanced patch was constant for each individual across the trials and counterbalanced between individuals. At the beginning of the trial, the enclosure was opened to allow the lizard to willingly enter a patch of its choice. In some instances, they were gently prodded at the base of the tail within the enclosure to make the animal orient towards the experimental arena, following which they were left to decide which patch they entered. Each trial lasted 30 min and was video recorded. After the experiment, the lizard was removed and all remaining crickets were removed from the foraging patch.

For the second objective, the enhanced patch was further enriched by a colour (orange or pink) to further enforce learning and to explore if lizards learn colour or position of infested crop patches.

Coding foraging behaviour

We scored the following variables i. time spent in the rewarded patch versus the non-rewarding patch ii. Correct choice made. We considered a choice as correct when the reptile chose to forage in the rewarding patch at first iii. Amount on pest consumed from the enhanced and non-enhanced patch, iv. latency to enter the enhanced patch, and v. number of switches between the patches.

Statistical analyses

We used Mann-Whitney's U test was used to explore time allocated by the lizards between the promising food patches compared to non-rewarding foraging ground, the number of correct choices made between correct and incorrect patches, food intake varied between rewarding and non-rewarding patches. We performed a series of generalized linear regression (GLM) to understand the factors that might be influencing food intake in the reptiles. Priliminary results are provided in the final evaluation sheet. **This work is under review**.

General methodology for colour and taste discrimination

Color association

Nine reptiles performed in this experiment. Eight of them have previously participated in colour discrimination and hence were not naïve to the experimental set-up. Each reptile was given 22 training sessions each of 10 min when mealworm was offered in a green container. Following the trial sessions each reptile participated in six tests during which two meal worms were offered simultaneously in two containers one blue and one green. Both the worms were mobile and active to ensure the worms in both containers elicited similar response from the lizards as Oriental garden lizards have been reported to be irresponsive towards immobile prey even in presence of prey odor (Shanbhag et al., 2010).

Experimental trials for associating colour with taste

We manipulated the taste by coating 0.2ml of Choloroquine phosphate following (Shanbhag et al., 2010). Previous study has shown garden lizards find Choloquine phosphate to be bitter however the chemical does not have any measurable toxic side effect (Shanbhag et al., 2010). Bitter and normal prey were made visually discriminable by placing them in green and blue containers placed such that both the worms were clearly visible to the lizards. Here the lizards had to form a novel association between taste and the green colour of the container.

Analyses

We considered a choice was made when either lizards fed only from green container or chose the prey in green container first. We compared the food intake and latency to approach either of the containers using Mann-Whitney's U test. To understand if reptiles can associate taste with colour, we used Man-

Whitney's U test on the total food intake, the frequency of food intake, and latency to approach blue and green containers with natural and bitter-tasting prey from green vs blue containers. We explored the possibility of repeated negative exposure influencing food choice for both natural and distasteful prey and whether latency was dependent on the number of trials, the colour of the container and if food intake varied with trial numbers by using series of Generalised Linear Model (GLM). Priliminary results are provided in the final evaluation sheet. **This work is under preparation and soon to be submitted**. For all experiments videos were coded to an accuracy of 0.001 seconds using software Boris (v.7.13.6-2022-5-18).statistical tests were performed using R software (version 2022.07.0). 20% of the videos were coded by another participant.

Habituation of reptiles to the study

Lizards were housed in their home enclosure for 1.5 months before they participated in any tests. During this time, we made sure that reptiles fed regularly and became accustomed to human presence as well as to the captive conditions. Captive conditions were monitored by a vet during the study. They were fed within their enclosure and had water available *ad libitum* to reduce stress from regular handling and thereby reduce or prevent stress related learning impairments (Langkilde & Shine, 2006).

Post-experiment activities

All lizards were finally checked for parasite load or any other disease that they might have contracted while in captivity by a vet. Reptiles were marked with wearable non-toxic felt pen on the lateral sides of their body for post release monitoring. Reptiles were released to new sites as their original habitats either had excessive human disturbance and many sites had been converted into apartments over the time period of the study. We performed a reccee of the release sites prior to release regarding the prey availability, predatory threats and human disturbance (refer to the photographs below). These sites were within 1 km of their original habitat and was similar in structure and composition.

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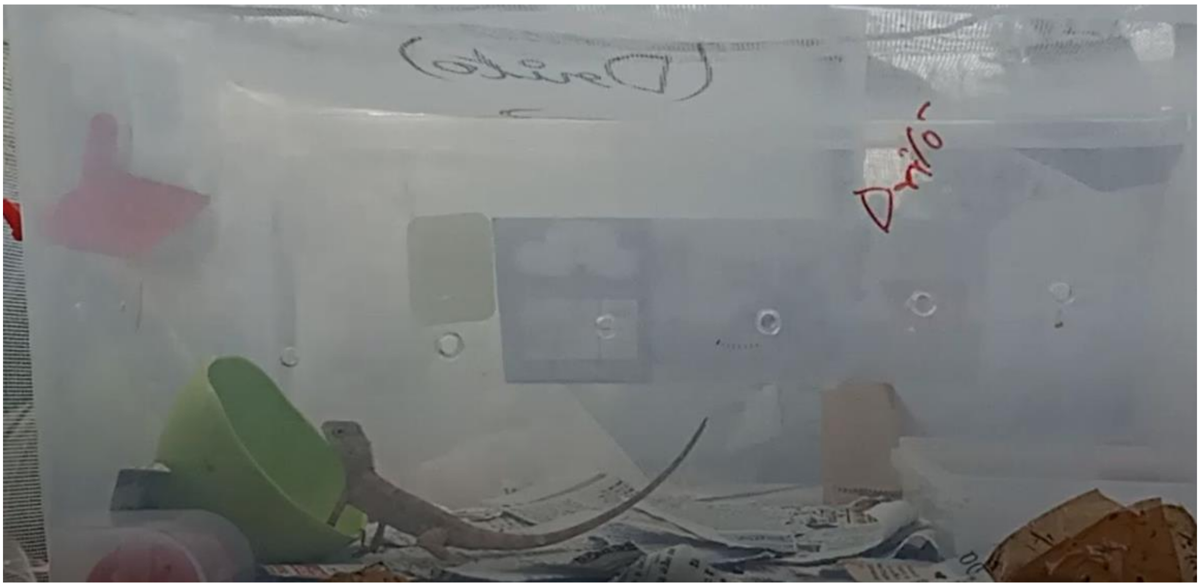
Photographs from the project

Initial enclosure construction



Colour association training

Colour association task

Reptile marking and release

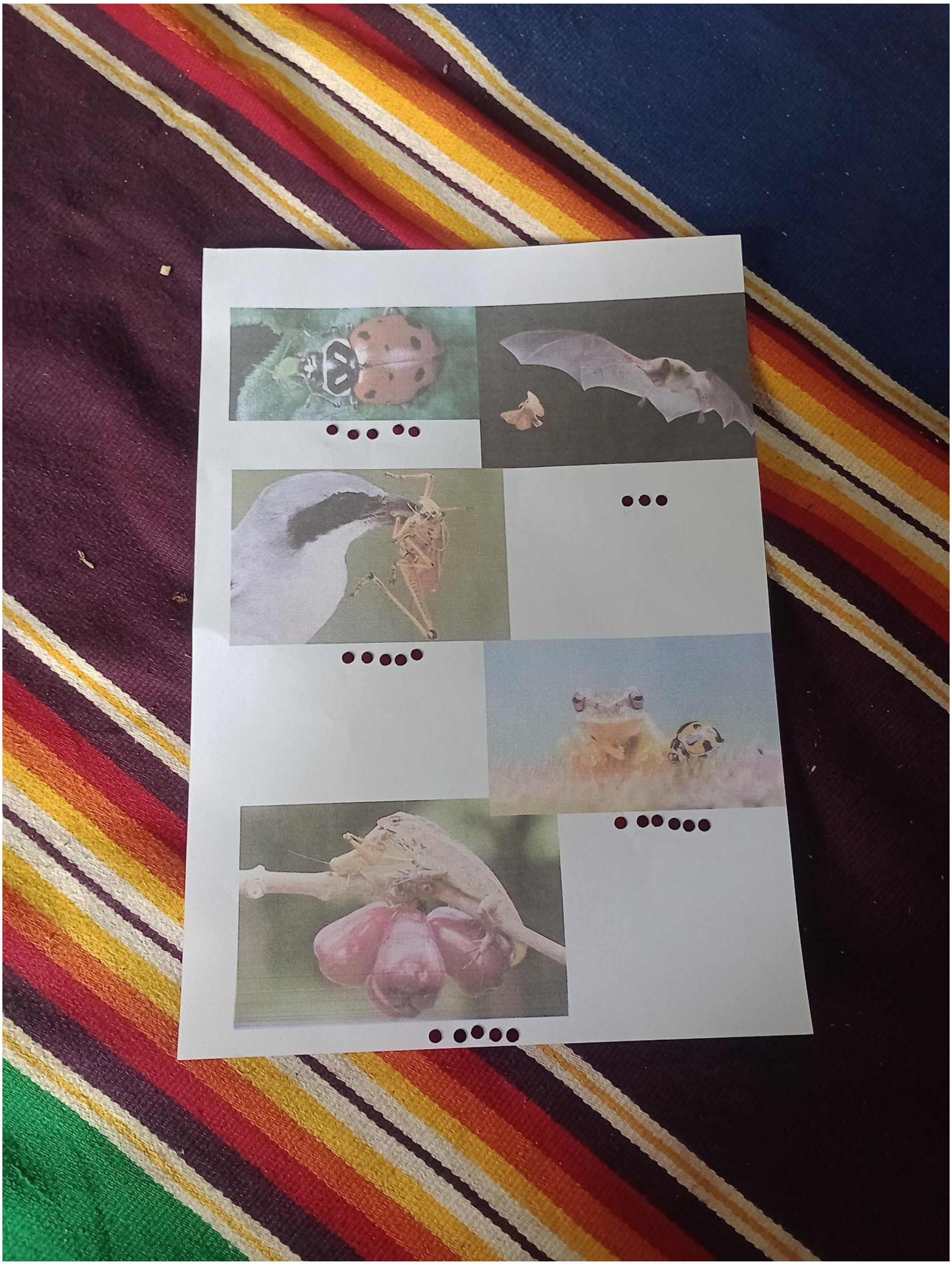
Marked reptile released in the wild

Reptile release team

Outreach programs and outcome dissemination of the project

I. Community outreach: Farmers engaged in activity

Farmers voting for animals they think to be important for agriculture



Voting sheet form one group of participating farmers

II. Field study session with Masters' students on reptile cognition behaviour and pest regulation

III. Conferences

Presenting at ASAB. Photocourtesy Dr. Kate Lessels, treasurer at ASAB

Presenting at BES. Photocourtesy Anoop NR

All the subjects who participated in the trial (created by Tushar)

Appendix

Health certificates

Dr. Karthik, M.

M.V.Sc. (Wildlife) *Mob:* +91 9035753717

KVC No.: 4604 *e-mail:* kart.rumble@gmail.com

To Whomsoever Concerned

Date: 02. 06. 2022

This is to certify that the following reptiles with the corresponding details were examined on this day.

Sl. No.	Name of the Reptile	Species / Common name	Comments
1.	Gabby		NAD
2.	Gulliver		NAD
3.	Grump		NAD
4.	Nova		NAD
5.	Tiger	*Calotes versicolor* /	NAD
6.	Spotty	Common Garden Lizard	NAD
7.	Pirate		NAD
8.	Naar		NAD
9.	Nari		NAD
10.	Hope		Advised Vermiculite in the housing

No clinically evident or significant abnormalities were noticed in any of the reptiles. All of them appeared in good health status with good body condition and appeared to be free of any demonstrable or apparent infections or diseases or disorders.

Dr. Karthik.M, M.V.Sc (Wildlife)
KVC No. 4604

Address: No. 10, "Manjushree", I Cross, VII Main (Corner), Chikkadugodi, DRC Post, Bangalore, Karnataka, India.
Pin code: 560029

Dr. Karthik, M.

M.V.Sc. (Wildlife) *Mob:* +91 9035753717

KVC No.: 4604 *e-mail:* kart.rumble@gmail.com

To Whomsoever Concerned

Date: 30. 07. 2022

This is to certify that the following reptiles with the corresponding details were examined on this day.

Sl. No.	Name of the Reptile	Species / Common name	Comments
1.	Gabby	*Calotes versicolor* / Common Garden Lizard	NAD
2.	Gulliver		NAD
3.	Grump		NAD
4.	Tiger		NAD
5.	Spotty		NAD
6.	Pirate		NAD
7.	Naar		NAD
8.	Nari		NAD
9.	Hope		NAD

No clinically evident or significant abnormalities were noticed in any of the reptiles and their health and body condition was visibly better than the previous examination. The temperature in the housing facility was maintained at an average of 24-25 degrees Celsius and humidity at an average of 60-70%. All individuals appeared to be in good health free of any demonstrable or apparent infections or diseases or disorders.

Dr. Karthik.M, M.V.Sc (Wildlife)
KVC No. 4604

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Pin code: 560029

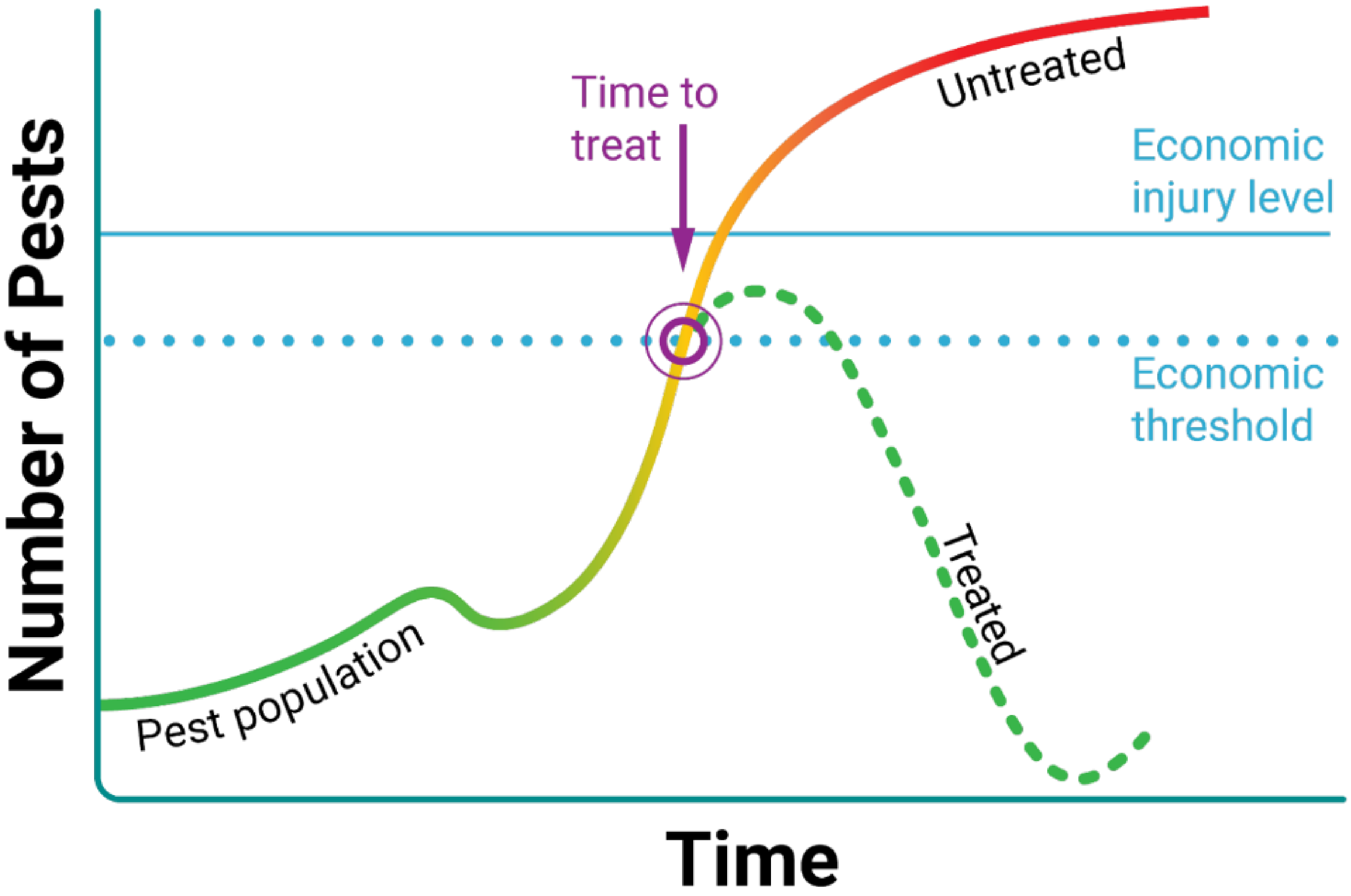
Why do we need our farmland biodiversity?

Deyatima Ghosh
Presented by Harisha

UNIVERSITY OF LINCOLN

The Rufford Foundation
www.rufford.org

atree



Number of Pests
Time to treat
Untreated
Economic injury level
Economic threshold
Treated
Pest population
Time

Why do we need biological pest control?

60% of India’s total geographical area is currently under cultivation

By **2050- 1,80,00,00,000 hectares**

Crop pest causes loss of 40%

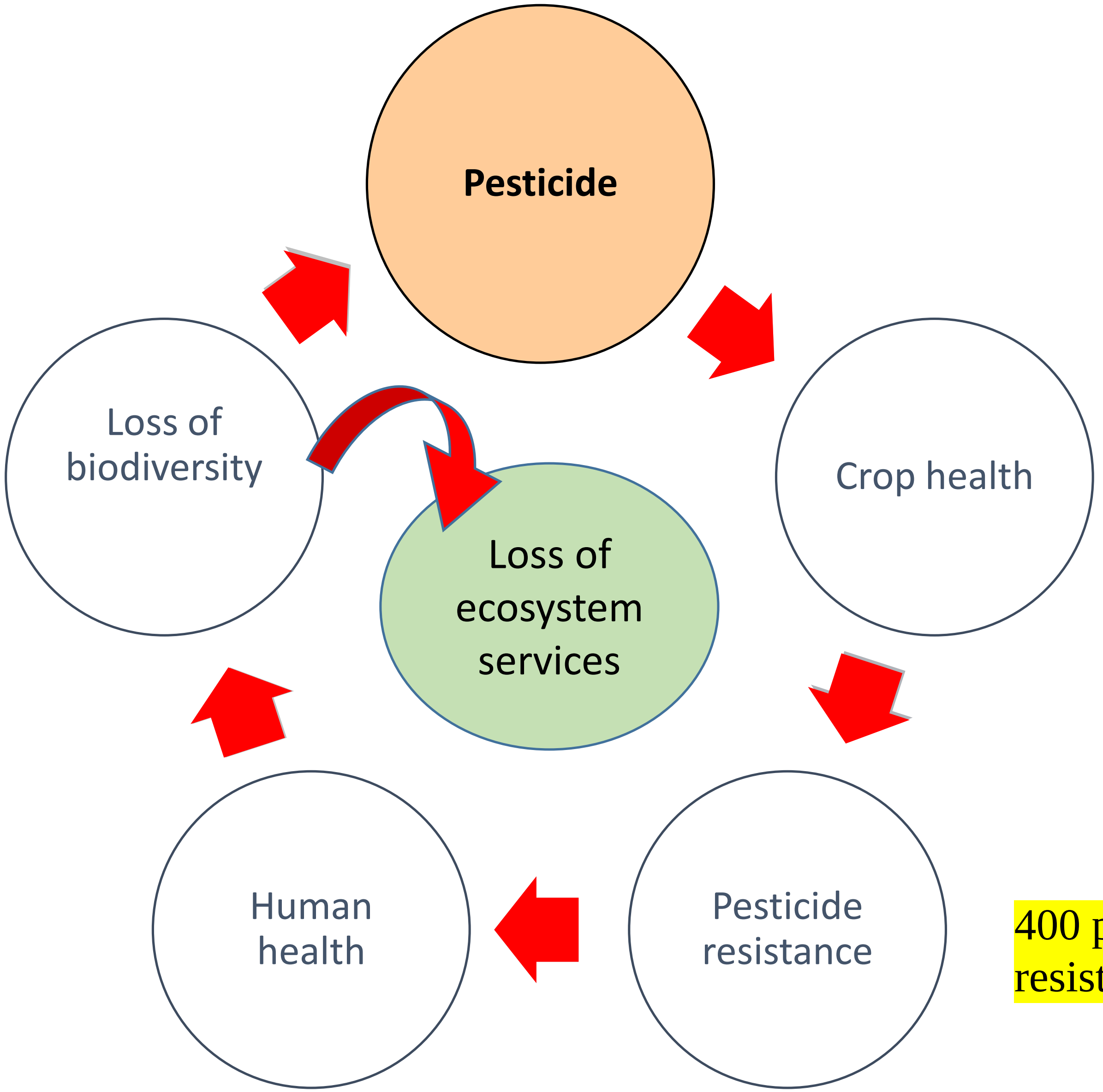
Economic loss- 3,84,37,54,00,00,000/-

Pesticide -3,000,000,000 Kg

Expense on pesticide- 32,71,28,00,00,000/-

400,000,000 Kg of active ingredients

What was the solution?

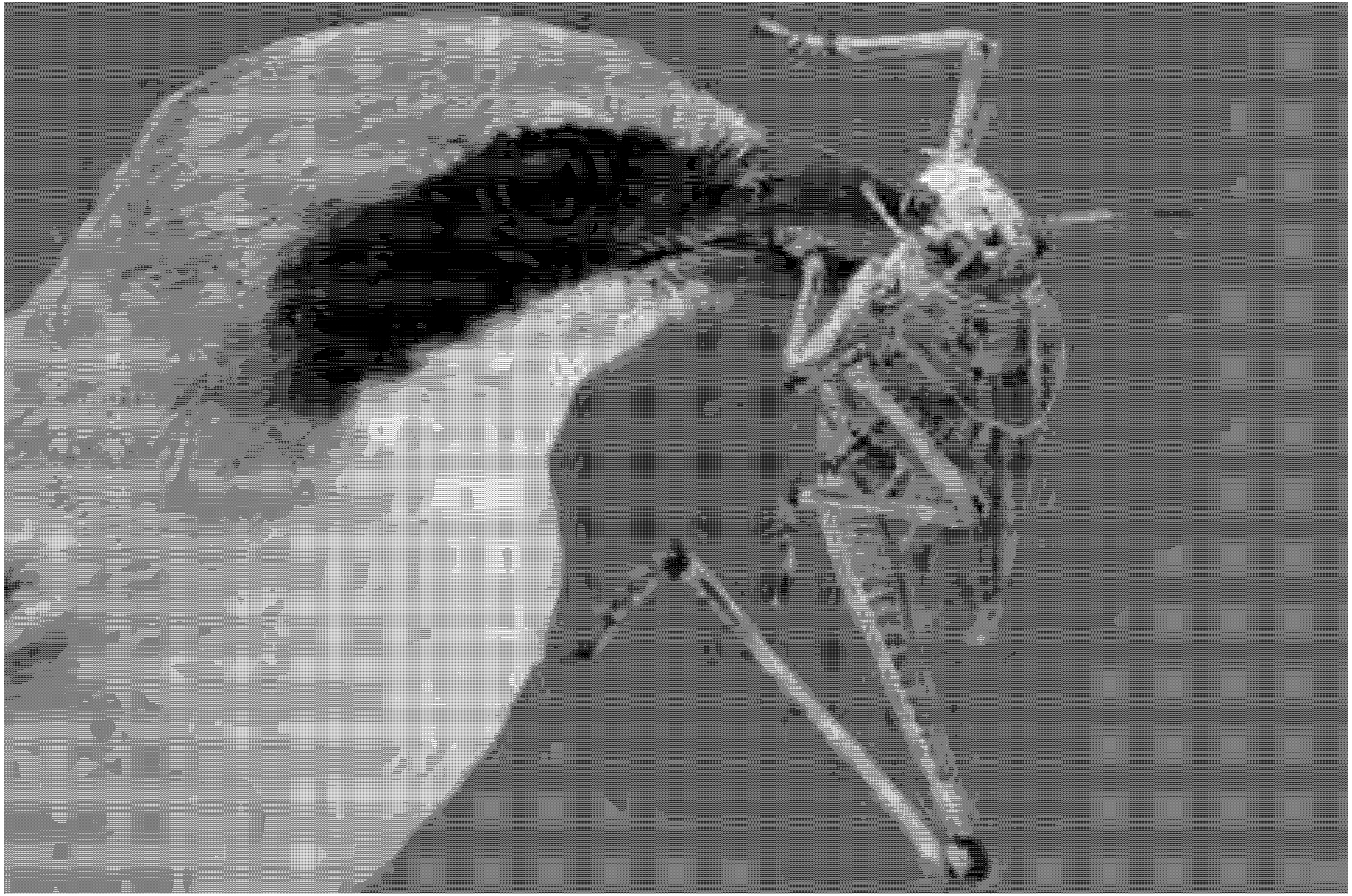


400 pest species have evolved resistance to one or more pesticides


T18P

How do the farm animals help us?

- 1 natural arthropod pest predator can bring a profit of Rs. 0.81
- Only arthropods cause 50-60% of biocontrol accounting to a profit of Rs. 3,25,89,16,00,00,000/-
- Birds cause an economic benefit of Rs. 25256.60/ha
- 150 Bats can consume 12 lakhs of pests in one year
- 46 to 83% reduction in pest over a 24-hour experimental trial
- In 30 min, Lizard can eat 10 crickets

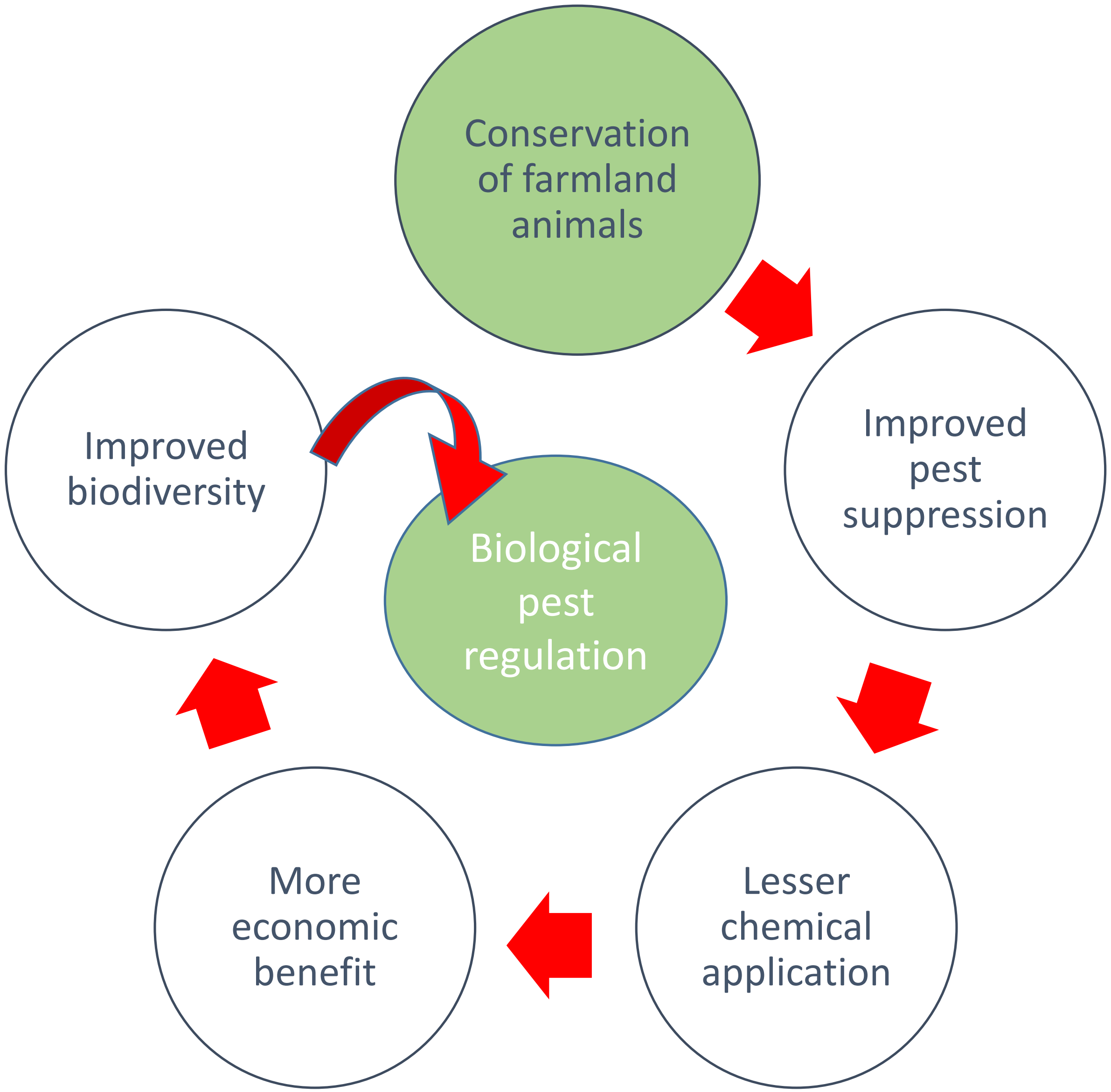



NAKODA

Reptiles are intelligent

- Reptiles can discriminate between more and less infested crop patches
- They choose the more infested patch
- They spend more time
- They feed on more crop pests
- They can remember the position of more infested crop patch
- Reptiles can discriminate between taste
- They can discriminate between color

How can we make a sustainable system?



How can we make a sustainable system?