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Smaller Body Mass Influence Less Sensitive to Neonicotinoids of Honey Bee Apis Cerana Indica; Fab

R Padmavathi[1], P Sethuraj[2]

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Abstract

Multiple stressors and interaction between them may be responsible for the decline of global pollinators. Among them, exposure to neonicotinoids has been getting more attention and has been considered as a main stressor. The Western honey bee (Apis mellifera L.) (Hymenoptera: Apidae) and indian indigenous honey bee (Apis cerana F.) (Hymenoptera: Apidae) are two managed honey bee species in India. These two species are widely used in beekeeping, and many wild A. cerana is widely spread in forests and contributes to the ecosystem. It is predicated that A. cerana is more sensitive to insecticides than A. mellifera due to their smaller mass. Here, we found that although the body mass of A. cerana is significantly lower than A. mellifera, the sensitivity of the two species to neonicotinoids are not associated with their body mass but depended on the chemical structure of neonicotinoids. To dinotefuran, the two species showed the similar sensitivity. To acetamiprid, A. mellifera was less sensitive than A. cerana. However, to imidacloprid and thiamethoxam, A. mellifera was more sensitive than A. cerana. These results suggested that the sensitivity of honey bees to neonicotinoids is closely associated with the structure of pesticides, but not with body mass of bees. It is also indicated that the hazards of pesticides to the different pollinators could not be inferred from one species to another.

Keywords: Apis Mellifera Linnaeus; Apis Cerana Fabricius; Neonicotinoids; Oral Acute Toxicity.

Introduction

Pollinators play an essential role in ecosystem services and global food security. Previous studies suggested that 75% of the world's leading food crops depend on animal pollination, furthermore, 35% of crops production rely on insect pollination to some extent (Klein et al. 2007).[3] With the development in agriculture and diet change in humans, the pollinator dependent crops have increased approximately threefold in the past 50 yrs (Klein et al. 2007, Breeze et al. 2011).[17] However, evidences showed that the population of honey bees decreased worldwide in recent decades (Goulson et al. 2015)[9] this decline may be attributed to many stressors, including parasites (Graystock et al. 2013)[10] habitat losses (Vanbergen et al. 2013), pesticides (Pisa et al. 2015)[26] and their interactions (Vidau et al. 2011). Among them, pesticides, especially neonicotinoids, have arguably received the most attention (Cresswell et al. 2012)[4] Goulson et al. 2015).

Neonicotinoids are neurotoxins that target

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the insect central nervous system, causing over stimulation, paralysis, and death (Matsuda et al. 2001).[23] For the high efficiency, wide spectrum, low vertebrate toxicity, and systemic of neonicotinoids, seven neonicotinoid insect cides such as imidacloprid, acetamiprid, nitenpyram, thiamethoxam, thiacloprid, clothianidin, and dinotefuran were commercially marketed (Bass et al. 2015)[2] It is also been reported that neonicotinoids occupied more than 25% of the pesticide market in 2014 (Bass et al. 2015)[2]. Due to widespread application, residues of these pesticides have been found in pollen, nectar, soil, guttation, and water, and these poisons have been considered as one of the main causes for the decline of the bees in worldwide (Mullin et al. 2010[26], Pisa et al. 2015).

There are over 8.95 million managed beehives in India mainland in 2014 (http://www.fao.org/faostat/en/#data), among them, Apis cerana F. and Apis mellifera L. (Hymenoptera: Apidae) are the two mainly managed honey bees. It is well known that these two species. differ in their morphological (Ruttner and Maul 1983), biochemical (Manzoor et al. 2013),[22] physiological, and behavioral traits (Tan et al. 2012).[38] For instance, A. cerana possessed a better olfactory sense than A. mellifera (Yang 2005)[44], and is more efficient in finding and pollinating the flowering plants scattered in the forest region, while A. mellifera hardly visits the sporadic plants growing in a secluded. place (Ji et al. 2003). In addition, the low and high limits of foraging temperature for A. cerana foragers is wider than A. mellifera, so that A. cerana can spend more time to pollinate the plants, it can also pollinate effectively at higher latitude with lower temperature (Tan et al. 2012).[38] Despite larger body and colony size, the pollination effect iveness of A. mellifera is not higher than A. cerana (Pudasaini and Thapa 2014).[39] In a word, these two managed honey bees play their irreplaceable roles in the ecosystem and agriculture (Ji et al. 2003).[15]

A previous study showed that A. mellifera is more resistant to commercial malathion, cypermethrin, demeton-s-methyl, fenvalerate, and deltamethrin than A. cerana for contact toxicities (Sharma and Abrol 2005), but it is not clear for oral acute toxicity. The wide application of neonicotinoid pesticides on crops and forests had negative impact on honey bees (Fairbrother et al. 2014)[7], although which species is more sensitive to neonicotinoids is not clear. We predicted that A. cerana is more sensitive than A. mellifera for their smaller body mass. Here, we explore the relationship between the body mass and their sensitivities to five neonicotinoids.

MATERIALS AND METHODS

Experimental Insects

The experiment was carried out on two species, A. mellifera and A. ceranas. To minimize the risk of disease, all the colonies were checked every week. Also, the honey bees were raised in the non cultivated areas, had no chance of exposure to the neonicotinoids and other pesticides, especially before and during the experiment. Three colonies were randomly selected as three replications for each species, and the colony strengths for each species were adjusted to be a colony with a mated queen, frames of capped brood, food reserves, and about 25,000 individuals of similar strengths before experiments.

Insecticides and Bioassay

All insecticides were obtained from India Pesticide Industry Association as technical grade. They were imidacloprid (purity 97.00%); thiamethoxam (purity 96.00%); dinotefuran (purity 95.00%); nitenpyram (purity 95.90%); and acetamiprid (purity 96.00%).

Preliminary experiments were undertaken to determine the maximum concentration (causing about 100% mortality) and the minimum concentration (the mortality rate was not significantly different from the untreated controls). Insecticides were diluted with acetone; then, different volumes of insecticide were added into 50% sucrose solution (w:w) to obtain five different concentrations of insecticide. The content of acetone concentration in all treatments (including the control) was adjusted to the same according to its maximum concentration in sugar solution (500 µl/100 g).

Test Procedures

All the tests were performed at the laboratory from July to August 2021. The hive entrance was blocked with a piece of hardware cloth and pollen foragers were obtained by a vacuum bee collector (Huang and Robinson 1996, Robinson and Vargo 1997). The bioassay procedure was referenced from the guideline methods 120 foragers were used in each replication with 6 concentrations, with 20 foragers per cage (20 x 20 x 30 cm) with the bottom and two opposing walls as wood and the other walls made of a gauze mesh (0.3 mm). The caged bees were placed in the incubator at 24.5 ±

0.5°C and 50% relative humidity. Experimental bees were collected at 9:00 am in the sunny days and starved for 2 hrs; then, the sucrose solution with acetone alone or with pesticide was provided ad libitum until the end of the test.

Some scientists pointed that the pollen nutrition affects the sensitivity of bees to pesticides (Wahl and Ulm 1983, Huang 2012).[42] Bees will take pollen and nectar in their natural condition; so, we use pollen and sugar solution as a diet to raise the test bees. Pollen was collected from the apiary which was placed in the forest and no chemical pesticides were used on forest vegetation at the pollen collection time according to the forestry bureau and beekeeper. Mortality was observed after 48 hr. Dose-response curves were replicated in three different colonies.

Body Mass of Foragers

The weight of the foragers was determined by a digital balance (Shimadzu, Auw120D). Foragers were collected in the morning to avoid collecting returning bees from orientation flying bees; the for agers were captured with a pair of forceps when they exited from the hive to collect the nectar or pollen. Each forager was collected in a preweighed Eppendorf tube (1.5 ml), and the total weight was determined again to obtain the net weight of the bee. In total, 161 foragers were used for each colony, and repeated in 3 colonies for each species.

Statistical Analyses

The LC values of A. mellifera and A. cerana were calculated by probit analysis (Finney 1971) using POLO-PC software. The difference between A. mellifera and A. cerana for five neonicotinoids and their mass were analyzed with T-tests, and the differences among the insecticides were analyzed with one way analysis of variance followed by least significant difference (LSD) tests with SPSS v16.0.

RESULTS

Insecticide Toxicities to Honey Bees

Table 1 shows the oral toxicities of five neonicotinoids against A. mellifera and A. cerana. The LC, value of acetamiprid to A. mellifera (353.36 µg/g) was significantly higher than thiamethoxam (8.23 µg/g). The LC values for A. mellifera to imidacloprid, dinotefuran, and nitenpyram were 2.90, 5.90, and 6.32 times higher than thiamethoxam, respectively. As well, the similar sensitivity trend was found in A. cerana; results showed that thiamethoxam is the most toxic pesticide in these five neonicotinoids, while the acetamiprid is the least toxic.

Body Mass of Foragers

The average body mass of A. cerana forager was 73.95

0.55 mg

(mean ± SE), significantly lower (T-test, P = 0.026) than that of A. mellifera (99.45 ± 0.70 mg) (Fig. 1).

Discussion

Neonicotinoids pose the largest risk to honey bees at a global scale, according to the toxicity data, residue detected frequency in hives and a comprehensive evaluation of risks (Sanchez-Bayo and Goka 2014).[33] While most studies were focused on the toxicity of neonicotinoids to A. mellifera, only a few studies demonstrated the effect of pesticides on A. cerana, Previous studies suggested that the risk of pesticide to each

Table 1: The toxicities (LC50 values) of five neonicotinoids against A. mellifera and A. cerana.

	Apis mellifera		Apis cerana		
	Mean ± SE (µg/g)	95% CI	Mean ± SE (µg/g)	95% CI	LC 50 Values comparison
Acetamiprid*	353.36 9.23[a]	313.64-393.08	236.02-334.60	236.02-334.60	A. mellifera > A. cerana
Dinotefuran	48.48 3.27[b]	34.40-62.56	34.88-66.50	34.88-66.50	A. mellifera = A. cerana
Nitenpyram*	52.14 ± 0.93[b]	48.15-56.12	57.36-111.60	57.36-111.60	A. mellifera < A. cerana
Imidacloprid*	24.09 + 1.19[c]	18.96 29.22	21.11-53.57	21.11-53.57	A. mellifera < A. cerana
Thiamethoxam*	8.23 ± 0.56[c]	5.82-10.64	11.29-21.61	11.29-21.61	A. mellifera < A. cerana

Statistics: Different lower case letters in same sub-column showed significant difference among the same honey bee specie to different neonicotinoids, $P < 0.05$ (one way analysis of variance followed by LSD tests); * showed significant difference between these two species to the same neonicotinoids, $P < 0.05$ (T-tests).

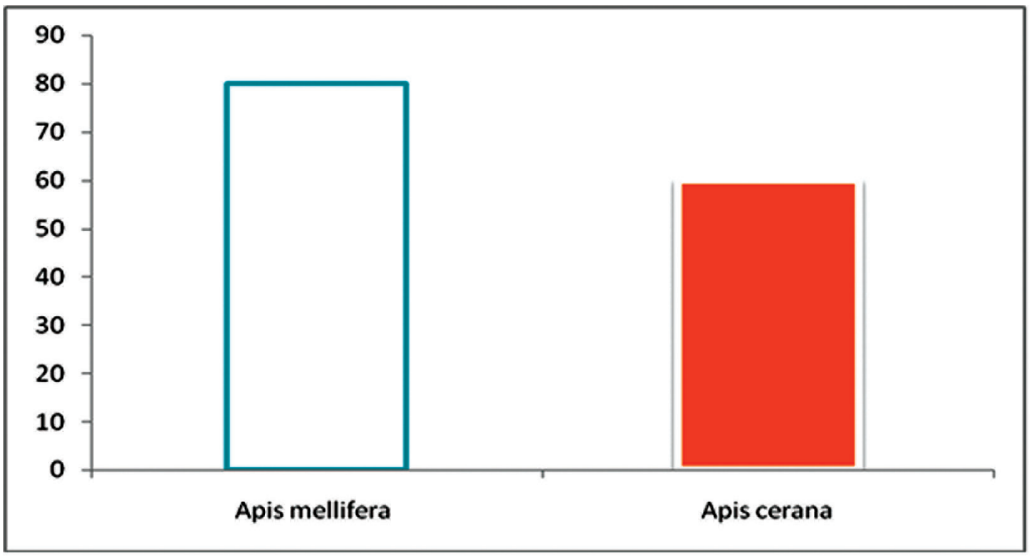

Fig. 1: The body mass of foragers in Apis mellifera and Apis cerana (T-tests, P = 0.026).

species of pollinators cannot be inferred from one species to another 1(Arena and Sgolastra 2014)[31], Rundlof et al. 2015, Moffat et al. 2016).[24] Furthermore, with the wide use of neonicotinoids in Indian, it is very necessary to evaluate the sensitivity of A. mellifera and A. cerana to neonicotinoids for the protection of honey bees.

Some studies demonstrate that A. mellifera is the most sensitive species or subspecies to insecticides. Laurino et al. (Laurino et al. 2013)[18] found that A. mellifera was more sensitive than other bees in neonicotinoids imidacloprid and thiamethoxam, the same results were previously found by (Danka et al. 1986)[5], they found that Africanized bees showed greater tolerance to azinphos-methyl, methyl parathion, and pyrethroid cyfluthrin than A. melli fera. Here, we found that the relative toxicity value of A. mellifera was higher for acetamiprid (1.2-fold). Nonetheless, no difference was observed in dinotefuran. On the other hand, A. cerana showed more resistance than A. mellifera for nitenpyram, imidacloprid, and thiamethoxam. It was suggested that different neonicotinoids had different toxicities to different honey bees; it also provided the information that A. cerana is less sensitivity to most neonicotinoids. These results supported that the toxicities of pesticides to different honey bees must be evaluated separately.

The sensitivity of honey bees to insecticides is a result of the concerted action of manifold factors. Four main factors were body mass (Thompson 2016)[39], genetic background, physiological characters of the honey bees, and the structure of the chemicals, respectively. First, the body mass of bees may be considered as one of the primary factors, although the relationship between the mass of bees and their sensitivities to pesticides was controversial. Some scientists reported that the heavier the insect, the lower its sensitivity to pesticides (Steen 1994[37], Devillers et al. 2003).[6] However, other scientists found that bees with larger mass maybe more sensitive to pesticides (Wu et al. 2010).[43] Here, we found that the body mass of A. mellifera is significantly larger than A. cerana, but A. cerana is not more sensitive than A. mellifera; it was suggested that the toxicity of pesticides to honey bees have no positive correlation with their body mass.

Another important mechanism is the genetic and physiological characters of the bees. Different kinds of bees are different in their genetic background, morphological, biochemical, physiological, and behavioral traits, and these differences make their different sensitivity to the same pesticide. Laurino et al. (Laurino et al. 2013)[18] found that the toxicities of imidacloprid to A. mellifera L. and A. carnica were different. Rinkevich et al. (Rinkevich et al. 2015)[29] also found that the different genetics

may be responsible for their toxicity differences. Many other physiological characters such as ages (Rinkevich et al. 2015)[29], resistant gene (Gregorc et al. 2012),[11] immune systems (Reeves 2013),[28] and detoxifying enzymes (Scott 1999) may contribute to the difference of the toxicities. Here, we found that A. mellifera is not always more resistant than A. cerana; we speculated that their different genetics and physiological characters may be respon sible for this difference partly.

CONCLUSION

Lastly, the sensitivity of the honey bees to pesticides may be related with the structure of the chemical compounds. As we know, thiamethoxam, imidacloprid, nitenpyram, and dinotefuran are nitro substituted compounds, but acetamiprid is a cyano-substituted neonicotinoid. Generally speaking, the nitro-substituted neonicotinoids are more toxic than cyanosubstituted neonicotinoids (Laurino et al. 2010). Our results showed that the toxicity of these five neonicotinoids to honey bees was in the following order: thiamethoxam > imidacloprid > dinotefuran > nitenpyram > acetamiprid. The result is consistent with the topical contact toxicity of acetamiprid, imidacloprid, and thiamethoxam in the laboratory test and tier II evaluation at their field recommended concentrations (Stanley et al. 2015).[36] The results also suggested that the toxicities of these neonicotinoids to these two honey bees may relate with the structure of insecticides tightly but not the body mass of bees. A previous study suggested that honey bees take less time to consume the same volume sugar solution but with more diflubenzuron (Gupta and Chandel 1995).[12] Kessler suggested that bees preferred solutions containing thiamethoxam, but the total sugar solution consumption did not affect on A. mellifera (Kessler et al. 2015)[16]. Consumption of sugar and pollen by bees was not measured in this study. We think that the consumption of pollen and sugar solution needs more studies.

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Original Article

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Sensitivity Analysis of Yield Parameters to Elevated Temperatures in DSSAT V4.7.5 Simulation Model For CV. Swarna of Rice Over Khordha Region of Odisha

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Abstract

The study was conducted in Khordha district of Odisha during kharif season of 2014-2019, to see the effect of climate change especially of temperature onyield, LAI and yield attributes of swarna rice variety. Mainly changes in maximum and average temperature i.e (0.5 – 4.0°C) and (0.5 – 3.0°C) respectively through weather modification window of DSSAT CERES Rice crop simulation model were tried for the anthesis stage (60-90 DAP) of the crop. The experiment was laid out as a split plot design and replicated three times. The results showed that the yield was decreasing with respect to increasing in temperature after threshold limit. The interaction of maximum & average temperature with grain yield was significant only for 1st date of sowing andthe rest 2nd & 3rd date of sowing showed non significant. The leaf area index (LAI) interaction with maximum and average temperature did not show significant for only 2nd date of sowing but in case of 1st and 3rd date of sowing it showed significant. Like wise, the interaction of physiological maturity days with average temperature showed significant for all three date of sowing, though the comparision between maximum temperature and physiological maturity days showed decreasing order but there was no significant relation. The other two yield attributes like number of panicles/m^2 and harvest index also showed decreasing order while interaction with maximum & average temperature but there was not significant.

Keywords: Dssat Ceres-Rice model; Climate change; Swarna; Yield; Yield Attributes; Sowing Date; Tempearture; Anthesis.

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Introduction

Rice as a cereal grain, it is most widely consumed staple food for a large part of the world's human population, especially in Asia. Odisha is one of the premier Rice producing state in India. Odisha produces about 4.47% of rice in India. Although about 2/3rd of total cropped area of Odisha is devoted to rice, and the total production is about 7.6 quintals/hectare. The Khordha district of Orissa comes under the North-Eastern ghats, has the geographical area of 2888 sqkm, where the Rice is cultivated under the area of 136.8 Ha with the production of 110.3 tonnes and yield of 806Kg/hectare. Khordha district contributes to 2.79% of total Rice area in Odisha. Swarna is the Mid early

& medium maturity duration, which yields better under low nitrogen levels, possess seed dormancy and it is the highly stable variety.

The Decision Support System for Agrotechnology Transfer (DSSAT) is a set of computer programs for simulating agricultural crop growth. The DSSAT software package comprises crop simulation models for over 42 crops. DSSAT models simulate growth, development, and yield of crops as a function of soil plant atmosphere management dynamics. The crop models require daily weather data, soil surface and profile information, detailed crop management and crop genetic information as input. DSSAT and its crop simulation models have been used for many applications ranging from on farm and precision management to regional assessments of impact of climate variability and climate change.

The optimum temperature for rice cultivation is between 25°C and 35°C. Any further increase in mean temperatures during sensitive stages may reduce rice yields drastically. In tropical regions, the temperature increase due to the climate change is probably near or above the optimum temperature range for the physiological activities of rice (Baker et al. 1992). Intemperate Regions, rice growth is impressed by limited period that favours its growth (Reyes et al. 2003). Increasing trend of daily maximum temperature may decrease the rice

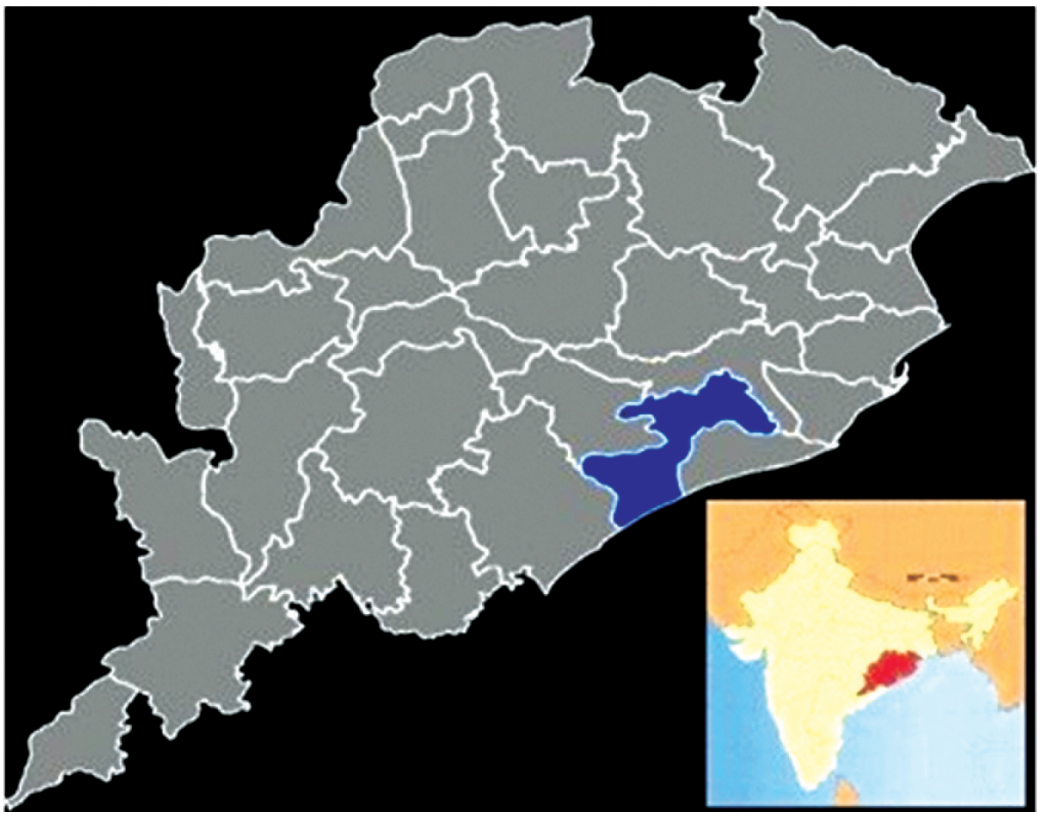

Fig. 1: Study area

spikelet fertility, which affects for reduction of the yield while the increasing trend of atmospheric CO_2 concentration could increase the rice yield (Dharmarathna et al. 2012). High temperatures would induce sterility and lead to low harvest index and grain yield.

Materials and Methods

Study Area: Khordha district of Odisha is selected as study area. Khordha is located at 20.18°N and 85.62°E, and the latitude and longitude of the district is 20. 1662379 and 85.6919708 respectively. It has an average elevation of 75m (246 ft). Area of the district is 28888 square kilometres (1115 square miles). It has the average temperatures of 41.4°C (Max) and 9.5°C (Min). and mean annual rainfall of

1443mm. The soils of Khordha district are classified under the Alfisols, Inceptisols, and Entisols. The amount of land suitable for rice cropping was 195,731 ha against the currently cultivated land of 122, 183.38 ha. Therefore, there was a possibility of more amount of land that could be available for rice cultivation in Khordha district.

Ceres-Rice Model

CERES is a process based, dynamic and mechanistic model which can simulate the growth and development of cereal crops under varying weather, soil and management levels. The various processes simulated by this model are phenological development of the crop; growth of leaves, stems and roots; biomass accumulation and partitioning among leaves, stem, panicle, grains and roots; soil water balance and water use by the crop; and soil nitrogen transformations and up take by the crop. This model is running under the DSSAT include the CERES (Crop Estimation through Resource and Environment Synthesis) for model cereal such as, rice, wheat, maize, sorghum, pearlmillet etc.

The weather data, soil data, and crop management data of about 6 years (2014-2019) and some required data of study area has been collected from the reports, websites, IMD and also by referring the research paper. Required Weather Parameters Like Maximum Temperature, minimum temperature, rainfall and solar radiation data taken from NASA Power website (https://power.larc.nasa.gov/data-access-viewer/) from the year 2014-2019. Layer wise soil data (0 – 80 cm) for Khordha district was taken from IMD, New Delhi. It includes so many soil parameters soil type, soil classification, slope percentage, runoff curve number, pH, bulk density, clay & sand percentage etc.

Table 1: General Information provided for Soil file in DSSAT.

Soil Classification	Alfisols
Color	Red
Runoff potential	Moderately low
Fertilit yfactor	1
Slope	1
Runoff curveno	84
Soil texture	Clayloam
Drain age type	Surface furrows

Crop management data: Crop management data which is required for DSSAT input from 2014-2019 taken from IMD. The data was used first calibration and then validation of the model. It consists of all the data from starting of field operation to harvest in gover rice crop in Khordha region. Planting Date of The Rice Crop For 1 St DOS, 2nd dos and 3 dos is 18 june, 18 july and 18 august respectively to study the climatic effect on crop.

Swarna is the Mid-early & medium maturity duration, semi-dwarfrice variety with the crop duration of 145-150 DAS which yields 4.5-5.5 t/ha under low nitrogen levels, possess seed dormancy and it is the highly stable variety.

Genetic Coefficient

Table 2: Genetic Coefficient of Swarna Rice Variety

Variety	P1	P2R	P5	P2O	G1	G2	G3	G4
Swarna	740	115	330.0	11.0	68	0.0213	1	1

These coefficients are crucial because they strongly influence the simulation of growth and development of the crop. The CERES-Rice model uses eight genetic coefficients viz., P1, P2O, P2R, P5, G1, G2, G3 and G4. The eight coefficients for the cultivar swarna are collected from IMD, New Delhi. The genetic coefficients for the cv. swarna are shown in Table 2 below as follows:

Methodology

Area as khordha district of odisha and swarna rice variety which is very popular in that region was taken. The average simulated yield and its parameters is taken as control and it is compare with the effect of maximum temperature and average temperature on the yield and its parameters for the year of 2014-2019.

Environmental modification: It is the part of DSSAT crop simulation model which help to modify or change the weather parameters as per requirements of the study. With the help of environmental modification solar radiation, maximum temperature, minimum temperature, rainfall, CO_2 concentration and humidity we can change on different time period or date. For the present study to see the effect of temperature on crop, there is increase in the maximum temperature

from 0.5 - 4.0°C and average temperature from 0.5 –3.0°C during anthesis period (60-90 DAP) in the crop simulation model.

Significance test: The Pearson correlation is the most widely used correlation statistic and linear regression analysis is used to measure the degree of the relationship between linearly related variables which is widely used in climate research, will be employed in this study to find out significance level of 0.05 and 0.01 (indicates 5% and 1% risk respectively) trends with the help of IBMSPSS statistics package.

Result and Discussion

Rice crop is more sensitive to temperature at anthesis period. So, to see the temperature effect on crop yield and its parameters, we did an analysis of grain yield, leaf area index at maturity, harvest index, maturity days and panicle number at three date of sowing which is listed below intable. The comparision between yield and temperature shows that the yield is decreasing with respect to increasing in temperature after certain limit. Similar results were obtained by Ray M. et al. (2018), which have reported that Increase in maximum and minimum temperatures beyond optimum temperatures forrice production led to a decrease in yield and minimum temperature changes had more profound negative impacts as compared to maximum temperature changes. The study revealed that increase in both maximum and minimum temperatures affects the grain yield. Similarly in Bangladesh, the impact of climate change on high yield rice varieties was studied by Karim et al. (1994), using the CERES rice model and several scenarios and sensitivity analysis. It was found that high temperatures reduced rice yields in all seasons in mostarid locations. But when we make regression analysis for the yield of three date of sowing with maximum and average temperature, only first date of sowing shows significance effect. Similar results have been reported by D. Rajalakshmi et al. (2015), that the maximum and minimum temperatures are projected to increase, while all other parameters indicated no consistent trend at the end of the century. The yield of rice is projected to decrease for both control and CO_2 enriched conditions. Likewise there also found significance between maximum temperature, average temperature and leaf area index (LAI) for first and third date of sowing. In the case of physiological maturity days with temperature it is found that the increase in maximum and average temperature results in decreasing the physiological maturity of the crop. By the regression analysis it is found that, there is only significance between average temperature with maturity days for three

Table 3: Model output of maximum temperature with yield, leaf area index, number of panicle, days to physiological maturity and harvest index for different date of sowing of cv. Swarna.

Temp. (°C)	Yield(kg/ha)			Leaf Area Index			Number of Panicle (no./m²)			Days to Physiological Maturity (DAP)			Harvest Index(%)		
	D1	D2	D3	D1	D2	D3	D1	D2	D3	D1	D2	D3	D1	D2	D3
Ctrl	3250.6	2907.1	3063.5	2.88	3.1	3.21	1055	1056	1056	96	94	98	39.6	34.8	30.5
Tmax+0.5	3205.8	2917.6	3085.5	2.86	3.1	3.26	1059	1056	1054	96	94	97	39.7	35.0	31.0
Tmax+1.0	3225.1	2929.3	3104.5	2.83	3.1	3.18	1060	1056	1053	95	94	98	40.2	35.1	30.9
Tmax+1.5	3237.8	2878.3	3119.8	2.83	3.1	3.25	1059	1057	1056	95	93	95	40.5	34.5	32.5
Tmax+2.0	3203.6	2893.3	3152	2.83	3.1	3.2	1058	1057	1054	95	93	98	40.3	34.8	31.2
Tmax+2.5	3142.5	2992.5	3060.8	2.78	3.08	3.25	1056	1058	1054	95	93	97	39.4	36.0	30.8
Tmax+3.0	3123.8	2990	3092.5	2.78	3.08	3.26	1056	1059	1061	95	93	97	39.4	36	31
Tmax+3.5	3095.6	2924.6	3221.3	2.76	3.06	3.23	1056	1060	1053	95	93	98	39.2	36.1	31.7
Tmax+4.0	3058.1	2932.1	3122.3	2.76	3.06	3.21	1055	1060	1055	95	93	94	38.9	36.4	32.5

(Tmax= Maximum temperature in°C)

Table 4: Model output of average temperature with yield, leaf area index, number of panicle, days to physiological maturity and harvest index for different date of sowing of cv. Swarna.

Temp.	Yield (kg/ha)			Leaf Area Index			Number of Panicle (no./m²)			Days to Physiological Maturity (DAP)			Harvest Index (%)		
	D1	D2	D3	D1	D2	D3	D1	D2	D3	D1	D2	D3	D1	D2	D3
Ctrl	3250.6	2907.1	3063.5	2.88	3.1	3.21	1055	1056	1056	96	94	98	39.6	34.8	30.5
Tavg+0.5	3127.8	2912.1	3110.1	2.8	3.1	3.18	1057	1057	1053	95	93	98	38.9	34.8	31
Tavg+1.0	3133.5	2853.6	3197.6	2.76	3.08	3.23	1056	1060	1053	95	93	98	39.3	34.6	31.6
Tavg+1.5	3055.3	2844.5	3051.1	2.75	3	3.23	1056	1057	1057	95	92	94	38.9	35.7	31.8
Tavg+2.0	3000.8	2790.1	2947.8	2.63	2.95	3.25	1059	1060	1057	94	92	93	39.3	35.6	32.1
Tavg+2.5	3038	2821.8	2950.5	2.65	3.05	3.26	1058	1061	1059	94	92	92	39.9	35.9	32.1
Tavg+3.0	3012.1	2781	2949.8	2.65	3.05	3.26	1057	1060	1059	94	91	92	39.8	35.8	32.4

(Tavg = Average Temperature In°C)

date of sowing. Number of panicles and harvest index also shows decreasing or draw it increases temperature. Though the set woparameters are showing decreasing trend line but there is no such significance seen. Similarly Wheeler et al. (2000), studies have also shown that even a few days of temperature above threshold value, if coincident with anthesis, can significantly reduce yield & yield attributes, through affecting subsequent reproductive processes.

Summary and conclusion

Temperature effect on yield and yield parameters has been analysed for 3 date of sowing of Swarna variety of Rice over Khordha district of Odisha. The analyses showed that the yield increases upto certain points and then tends to decrease with temperature and when the regression analyses was done with SPSS, the significance for 1st date of sowing has been shown, though the yield for rest 2nd and 3rd date of sowing is in decreasing trend but there was no significance. By Analyzing the data of Leaf area index with changing weather for anthesis stage of crop using SPSS software for significance test, the results are presented in the table 5 showed that the 1st and 3rd date of sowing were significant. The physiological Maturity Days was also analysed and the relation between the average temperature and Maturity Days of all 1st, 2nd, and 3rd date of

Table 5: Regression analysis of maximum temperature, average temperature withgrain yield, leaf area index and physiological maturity.

Temp	Grain yield			Leaf area index			Physiological maturity		
	D1	D2	D3	D1	D2	D3	D1	D2	D3
Tmax	R = 0.311Sig = 0.022* Y = - 40.988x 4495.595	NS	NS	R = 0.335Sig = 0.013* Y = -0.031x 3.816	NS	R = 0.277Sig = 0.042* Y = 0.024x + 2.515	NS	NS	NS
Tavg	R = 0.419 Sig = 0.006** Y = -70.988x + 5188.499	NS	NS	R = 0.406 Sig = 0.008** Y = -0.079x + 5.082	NS	R = 0.346Sig = 0.025* Y = 0.031x + 2.438	R = -0.484 Sig = 0.001** Y = -0.552x + 111.460	R = -0.338Sig = 0.028* Y = -0.507x + 107.479	R = -0.587 Sig = 0.00** Y = -1.834x + 142.520

(Tmax= Maximum temperatue, Tavg = Average temperature, R = Coefficient of correlation, *= 5% level of significance &**= 1% level of significance, NS = Non significant)

sowing was shown significance. The other yield attributes like number of panicles (per m^2) & harvest index (%) was also in decreasing trend with increasing temperature but the reisno such significance seen. The table 5 listed below shows the levels of significance, regression coefficient and regression equation for three date of sowing of swarnarice variety. Therefore to improve rice production with cv. swarna, always the optimum planting date should be used, proper understanding of the prevailing weather conditions and regular monitoring is necessary.

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Wild Bees as Environmental Indicators and Monitoring Agricultural Ecosystem: A Review

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Abstract

Wild bees are abundant in agricultural ecosystems and contribute significantly to the pollination of many crops. The specialisation of many wild bees on particular nesting sites and food resources makes them sensitive to changing habitat conditions. Therefore wild bees are important indicators for environmental impact assessments. Long term monitoring schemes to measure changes of wild bee communities in agricultural ecosystems are currently lacking. Here we suggest a highly standardized monitoring approach which combines transect walks and pan traps (bowls). The combination of these two methods provides high sample coverage and reveals data on plant pollinator interactions. We point out that comprehensive methodical, biological and taxonomical expertise is mandatory. The suggested approach is applicable to diverse monitoring goals in an agricultural context e.g. the impact of land use changes as well as monitoring potential effects of GM crops on wild bees.

Keywords: Wild Bees; Standardised Ecological Assessment; Agricultural Ecosystems.

Introduction

There are more than 2000 species of bee in Europe (Fauna Europaea (2011)[20] with a gradient in bee species diversity from the south (high) to the north (low) and from the east (high) to the west (low). This gradient is caused mainly by the climatic requirements of bees; most bee species are associated with sunny and warm locations[85] (Westrich 1989, Michener (2007)[53] About 750 bee species are found in Central Europe (Amiet and Krebs (2012).[3]

Bees have a keystone function in ecosystems (see Kratochwil 2003)[43] Pollination by bees is essential for the reproduction of many wild plants. In agricultural ecosystems, bees contribute to the pollination of many crops (Roubik 1995, Allen-Wardell et al. 1998)[1] Buchmann and Asher (2005)[11] and a correlation between bee diversity and the ecosystem service of pollination in agro ecosystems has been demonstrated in several studies (reviewed in Ricketts et al. 2008)[63] Pollination increases the yield and the quality of many agricultural field crops. A decline of bees could result in a reduced diversity of insect pollinated plants

(reviewed in Klein et al. 2007)[40] Moreover pollination of certain fruits can increase their micronutrient content (Eilers et al. 2011)[19] In Europe the value of insect pollination has recently been

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estimated at 15 billion Euros per annum (European Commission 2011).[20]

Bee species other than the domesticated honey bee are regarded as wild bees. Many species of wild bees are highly specialized on particular nesting sites and food resources (see [85]Westrich 1996, Michener (2007).[53] Thus wild bees are highly sensitive to anthropogenic ally driven habitat degradation and habitat fragment ation Brown and Paxton (2009)[10] Many wild bee species are considered as good indicators to evaluate the conservation status of open landscape biotopes (e.g. Schwenninger 1992, Schmid-Egger (1994),[70] Tscharntke et al. (1998)[79] Plachter et al. 2002,[61] Sepp et al. (2004),[73] Jauker et al. (2009)[36]

Life History Traits of Bees

Wild bees show a huge diversity of life history characteristics (Westrich 1989, Murray et al. 2009)[85] They can be grouped in accordance to their degree of ecological specialisation:

Nesting

About 50 percent of all bee species nest in a burrow in the ground [(e.g. sand bees (Andrena), sweat bees (Halictus/Lasioglossum)], favouring south facing banks and sparsely vegetated areas. Other bees nest in cavities such as borings in dead wood, small holes in walls or empty snail shells [e.g. mason bees (Osmia) or leafcutter bees (Megachile) or use old mouse nests or other cavities in the ground (e.g. bumble bees Bombus)].

Foraging

Adult bees generally use flowers of diverse plant species as nectar sources. Bee larvae develop on a diet of pollen and nectar (in Europe, only larvae of the genus Macropis are known to use floral oils as a substitute for nectar). The adult females provide their brood cells with a mix of pollen and nectar. Polylectic species are able to use pollen from a wide range of different plant families. In contrast, about 35 percent of the bees in Central Europe the so called oligolectic bees are highly specialized, collecting pollen only from certain closely related plant species (Zurbuchen and Muller 2012).[90]

Life Cycle and Sociality

More than 80 percent of European bee species are solitary. Females of these bees construct their own nests and provide food for their offspring themselves. Adult females usually only live for 4 to 6 weeks. In contrast, eusocial bees live in colonies. In Europe most bumblebees and many sweat bees are primitively eusocial. Their annual colonies are usually founded by a single queen. Queens of the eusocial sweat bee Lasioglossum marginatum live for 5 years. The only so-called complex eusocial bee species in Europe is the honey bee Apis mellifera; queens live up to 5 years, whereupon the colony's old queen is replaced by a daughter queen i.e. the colony is perennial.

Cuckoo Bees

About 25% of European bee species use the nests and provisions of a host bee species for their reproduction. These cleptoparasites "cuckoo bees" are usually associated with specific host species. Female parasites lay their eggs in the brood cells constructed and provisioned by the host female. In the case of parasites of social hosts, parasite females take over both host colonies and host workers. Usually parasite females kill the host queen. Thereafter the eggs, larvae and offspring of the social parasite are provisioned by the workers of the original host queen.

Wild Bees in Agricultural Ecosystems

Until the beginning of the 20th century, extensive farming practices such as three field crop rotation shaped landscapes and created diverse habitat mosaics. As a result of the industrial revolution, historical forms of land use changed rapidly (e.g. Kaule [37] 1991, Plachter (1991, Benton et al. 2003). The intensification of agriculture reduced the availability of foraging habitats and nesting sites for bees. For example, in arable regions, the loss of non-cropped areas and Fabaceae-rich grassland as well as the tendency to rotation crops lacking flowering cultivars decreased the habitat quality of these regions for bees (see Williams and Carreck (1994)[89] Goulson et al. (2005)[24] Bommarco et al. 2012)[9]

Wild bee diversity in arable landscapes is affected by the spatial and temporal avail ability of food sources and the presence of suitable nesting sites (e.g. Banaszak (1996)[5] Steffan-Dewenter (1998)[74] Tscharntke et al. 2005, Holzschuh et al. (2007).[34] Landscape scale factors as well as field scale factors likely influence the composition of bee communities in such ecosystems (Fig. 1).

For example non-crop habitats in the vicinity of farm sites can increase the species richness and abundance of bees (e.g. Kremen et al. 2004)[44]

Farming practices such as weed control and till age as well as crop rotation influence the quality of arable fields as bee foraging sites or nesting habitats. Several studies indicate a positive impact of e.g. fallows or certain flowering crops on bee diversity (Schwenninger 1992)[71], Gathmann 1998, Steffan-Dewenter 1998, Herrmann 2000, Saure et al. (2003)[67] Saure and Berger (2006),[68] Berger and Pfeffer (2011) Schindler and Wittmann (2011)[69] However, deteriorating habitat quality and the loss of habitat heterogeneity has led to widespread decrease in bee diversity and bee abundance in agricultural ecosystems. Most bee species need food and nesting resources close to each other sincethey have small activity ranges (Walther-Hellwig and Frankl (2000)[82] Gathmann and Tscharntke 2002, Greenleaf et al. (2007)[25] Zurbuchen et al. (2010b)[91] For this reason, the spatial and temporal availability of the resources can markedly affect the rate of reproduction of bees (see Zurbuchen et al. 2010a)[92]

An analysis of 23 studies of wild bee communities across different agricultural landscapes in Central Europe revealed a total of 293 bee species (Saure et al.[67] Only 54 of these bee species were found in more than 10 studies (see Table 1). These species are predominantly generalists (examples are shown in Fig. 2). According to the Red Data List of Germany, only three species (Andrena pilipes agg., Lasioglossum quadrinotatum, Bombus ruderarius) are endangered (Westrich et al. 2012),[87] two species (Colletes daviesanus, Melitta leporina) demonstrate floral specialisation.[87]

GM Crop Effects on Bees

Commercialized genetically modified (GM) crops could affect bee communities in two different ways, directly and indirectly (Table 2). GM crops carrying herbicide resistance (HR) and crops expressing insecticidal proteins derived from the bacterium Bacillus thuringiensis (Bt) are the most cultivated GM crops worldwide (James 2010)[35] Across a number of studies on honey bees, direct insecticidal influence of Bt-crops has never been reported (Duan et al. (2008)[16] These reports of nontoxicity are consistent with risk assessment data of Bt-crops on social and solitary wild bees (Malone and

Burgess (2009)[7] Nevertheless, only few studies address direct toxicity effects on this group of

Table 1: Number of bee species in agricultural landscapes (according to 23 evaluated studies, Saure et al. unpublished).

Taxonomic range	Infrequent (1-4 mentions)	Occasional (5-9 mentions)	Frequent (10-23 mentions)	Total
Colletidae	15	9	2	26
Halictidae	41	18	18	77
Andrenidae	31	18	18	67
Melittidae	5	1	1	7
Megachilidae	33	12	1	46
Apidae	36	20	14	70
Total	161 (55%)	78 (27%)	54 (18%)	293 (100%)

Table 2: Categories of potential effects of genetically modified crops on bees (reviewed in Morandin 2008).

Direct toxic effects	Indirect agroecosystem effects
	Unintentional alteration of the modified plant or differences in agricultural practices associated with the GM cultivar.
Toxicity of proteins expressed by the inserted gene on bees. Effects can lead to modified behaviour of bees. or can be sub lethal or lethal.	• Effects on the quality or attractiveness of foraging plants by altering the phenotype or physiology of the plant. • Effects on the foraging habitat by decreased weed abundance in and around the GM fields.

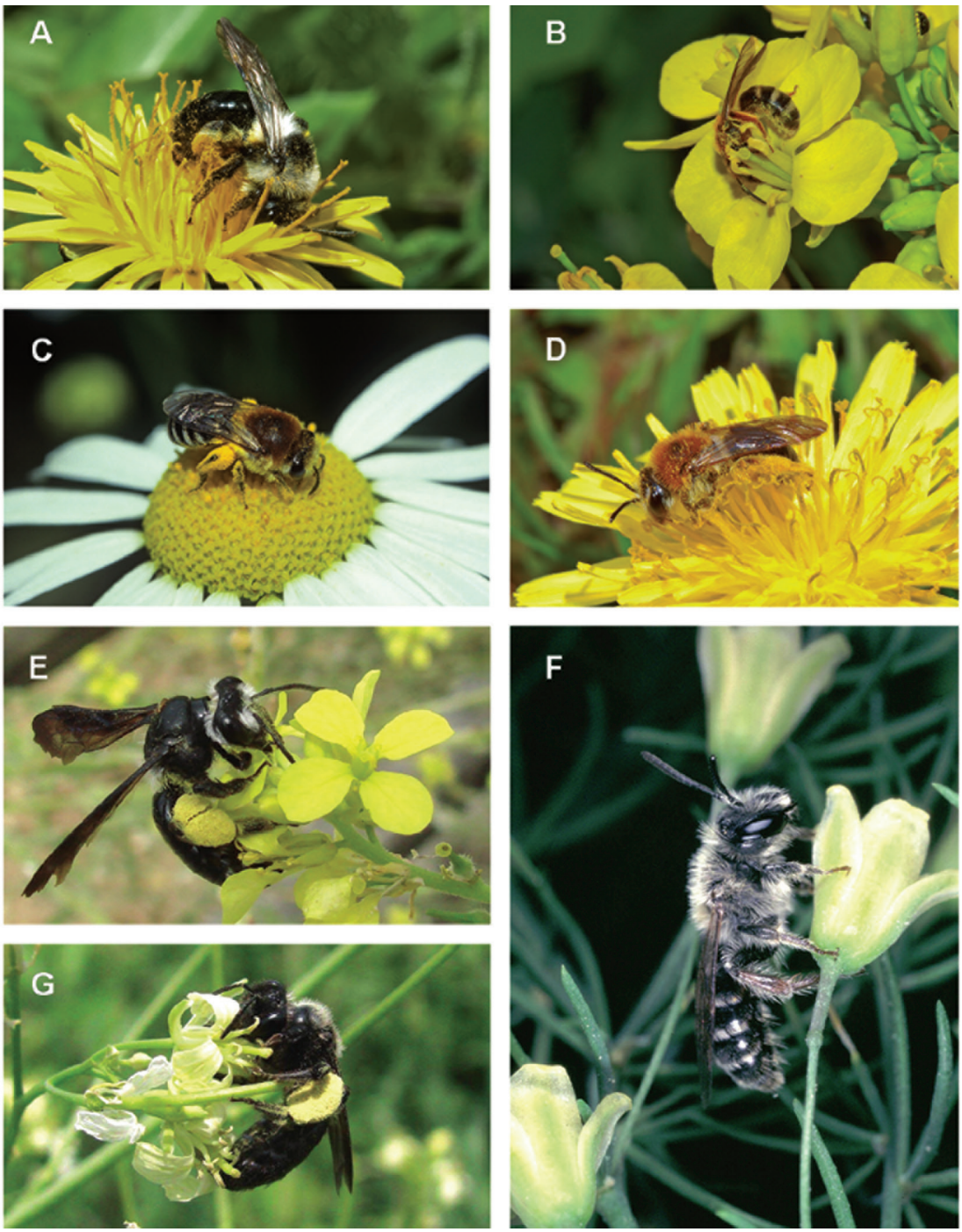

Fig. 1: Wild bee species which can be found in agricultural landscapes, **A:** *Andrena cineraria* (frequent), **B:** *Lasioglossum calceatum* (frequent) **C:** *Colletes daviesanus* (frequent), **D:** *Andrena haemorrhoa* (frequent) **E:** *Andrena agilissima* (infrequent) **F:** *Andrena chrysopus* (infrequent) **G:** *Andrena nigrospina* (occasional). *Fotos*: A, B, C, D: *Schindler*; E, G: *Diestelhorst*; F: *Schwenninger*.

pollinators. Exposed bumblebee colonies (Bombus impatiens, B. occidentalis, B. terrestris) did not display any effect of insecticidal Bt treatment (lethal or sublethal) (Morandin and Winston 2003; Malone et al. (2007)[50] Babendreier et al. (2008)[4]. Konrad et al. (2008, 2009)[41] did not find adverse effects of purified Bt-toxin (Cry1Ab) on life history parameters and on the longevity of the solitary bee Osmia bicornis. Currently, there are no indications that commercialized Bt-crops pose a direct risk to

bees. Nevertheless, new GM varieties have to be tested for direct effects on honeybees and wild bees.

Indirect effects of HR crops on bee diversity are most likely to occur. Only very few studies have compared agroecosystems having GM crops to other cropping systems in terms of their effects on wild bee populations. Different cropping methods associated with certain cropping systems such as field size and pesticide use may affect wild bee abundance (Morandin and Winston (2005).[56] Effective weed control through the application of broad-spectrum herbicides in HR cropping systems is suggested as a main factor influencing bee abundance (see Haughton et al. 2003),[28] Hawes et al. (2003)[29] Indeed, food resource availability plays a central role in regulating wild bee populations (Roulston and Goodell (2011)[66] Consequently, indirect effects of HR cropping systems on wild bees, including the application of broad spectrum herbicides, should be tested in GMO monitoring schemes.

Standardised Monitoring of Wild Bees

Entomologists have established numerous methods to monitor flower visiting insects such as bees, and several standardized methodical approaches have been developed (Table 3). A general description of methods has been published by e.g. Muhlenberg (1993),[57] Duelli et al. (1999)[17] and Sutherland (2010).[77] Schwenninger (1994) and Weber (1999) published detailed methodical specifications concerning inventory studies on wild bees. Westphal et al. (2008)[84] discussed methods,

Table 3: Common field ecological methods to survey bee diversity and abundance (adopted from Stey skal et al. 1986 and Sutherland 2010).

	Description of the method
Sightings/collecting with insect nets	Direct searching of bees e.g. at nesting sites or forage plants. This can be done by standardizing the area and/or the level of sampling effort. Bees are collected with insect nets.
Malaise traps	Malaise traps are made out of fine netting with vertical black screens and a bright sloping roof that leads to a collecting device (e.g. a jar filled with conserving agent). Flying bees hit the screen and walk upwards. Positive phototaxis (response to a light stimulus) of bees leads them to the bright roof and finally to the jar.
Window traps (Interception traps)	Window (acrylic glass) traps collect bees on the wing. Bee specimens fly into the window and drop into a bowl with water or with a liquid conserving agent.
Pan traps (Water traps, 'Moerike-traps')	Bee specimens fly into a pan or bowl with water or with a liquid conserving agent. The spectral properties of pan traps attract bees: yellow, white and blue pan traps are most suitable whilst UV-reflective surfaces enhance their attractiveness for bees.

which have been used to assess pollinator diversity and abundance.

Standardised monitoring of wild bees requires a methodological design which ensures a high sample coverage but minimizes significant negative impacts on population sizes of individual bee species. Monitoring collaborators should be highly familiar with the taxonomy, biology and ecology of bees. Furthermore basic knowledge of botany and methodical approaches for bee collection are essential.

Methodical Approach For Monitoring of Bees

Field studies relating to bee-monitoring preferably should be conducted for at least 3 years. To correct for temporal dynamics of wild bee populations across multiple years, the monitoring year must be regarded as random factor in the statistical analysis. In search of the most suitable methods for the monitoring of wild bees, one has to consider that negative effects of the studies on bee populations must be minimized.

For the monitoring of bees, we suggest a combination of sightings and collecting with insect nets along transect walks and short-term collecting with pan traps (max.one day). The use of temporarily positioned pan traps as a supplementary method may improve the results (see Meissle et al. 2012)[52]

Study Design

In each study area, adequate unploughed strips should be identified as study sites. On the unploughed strips at least 4 transects with a total length of 1000 m and width of 1 m should be defined. Transects should be established at representative strips within a radius (buffer) of 500 m from the borders of the target fields.

Next to each monitoring site, a 'control' or reference site should be identified at a minimum distance of 4 to 5 kilometres. This distance prevents overlapping of foraging ranges from monitoring to control site for e.g. bumblebees; some bumblebee species fly distances up to 2.5 kilometres (Walther-Hellwig and Frankl 2000)[82], Hagen et al. (2011)[27] The reference site should be situated in the vicinity of the study area. Landscape characteristics, farming practices as well as abiotic parameters in the reference site should be consistent with the study area. The choice of and sampling at transects should be conducted in the same way as in the study area. In cases where there are two observers per study, the observer of the study area must swap with the observers of the reference area and vice versa to avoid systematic monitoring biases. The advocated design, a paired design, allows for variation across large geographic distances for inherent differences in bee species diversity and bee abundance due, for example, to climate. A non-paired design would also be suitable, but at the cost of reduced statistical power; considerably greater number of sites would be required in a non-paired design to allow the effects of geography on bee species diversity and bee abundance to be partitioned out of a multivariate analysis of the impact of a specific anthropogenic factor (e.g. pesticide use) on wild bee communities Buhler (2012)[12]

Sampling of Wild Bees

The flight periods of bees are synchronised with the growing season of plants. Therefore field studies should be conducted monthly from mid-March to mid-September. The beginning of the sampling period can vary depending on the climatic conditions in different regions or years.

In March and April transect walks (50 minutes/250m) should be conducted between 10:00 a.m. and 4:00 p.m. In May-September transect walks (50 minutes/250 m) should be carried out both in the morning and in the afternoon between 9:00 a.m. and 5:00 p.m. Studies should be conducted only on sunny and windless days. Sampling should be repeated six times per year (every 3-4 weeks) at each study site. For each study site, the schedule and defined habitat parameters such as habitat type, abundance of habitats and size of habitats should be accurately documented on a standardised field report.

Bee specimens that can be identified directly in the field without catching should be registered in the field report. Those specimens that are difficult to distinguish should be collected with an insect net and stored in clear vials in a dark bag chilled with an ice package. Each vial should be labelled (e.g. location, date, time, number of the transect, host plant). At the end of the transect walks, bees collected in vials should be determined. Identified specimens should be documented by a macro photo as a voucher before they are release Unidentified wild bees should be exposed to ethyl acetate fumes. If DNA analysis (e.g. DNA barcoding) is necessary to identify specimens, bees should be exposed to ethyl acetate as short as possible in order to prevent degradation of the DNA (see Magnacca and Brown (2012).[48]

Before starting with the transect walks, one set of three UV reflective pan traps (yellow, blue, white) (Stephen and Rao (2005)[75], Droege (2006)[14] should be positioned in one representative transect at the level of the surrounding vegetation. The outer casing of the pan traps generally should be black to prevent attracting bees over a great distance. Pan traps should be filled with water and a drop of unscented detergent should be added as wetting agent. The pan traps should be removed at the end of the examination day.

The described method should also be used on crops that are potential foraging habitats for wild bees. A minimum of three surveys should be conducted during the crop flowering period. Pan traps should be positioned within the crop field and preferably at the same height as flowers of the crop. The distance of the pan traps to the field boundary should be at least 5 m to prevent attracting bees from non-crop areas (Droege et al. 2010).[15]

All collected bee specimens should be pinned with stainless steel insect pins. Wild bees should be prepared as described in Ebmer (2010).[18] Voucher specimens should be preserved in close fitting insect boxes. Samples for DNA analysis should be stored under cool conditions (e.g. a domestic fridge, +4°C) in ≥95% ethanol.

Analysis should be made by comparing the variables species diversity and species abundance of the anthropogenically altered sites (e.g. GMO monitoring sites) with their paired control sites (e.g.

GMO-free monitoring sites). The variance in count data may be addressed by either incorporating a comparison between e.g. GMO and e.g. non-GMO sites in the monitoring design or by analyzing additional data on the relevant environmental context (Lang and Buhler (2012).[47] A recent study corroborates local environmental factors such as habitat type, nesting resources and grazing regime as powerful determinants of community composition in bees (Murray et al. 2012).[58]

Environmental Monitoring with Wild Bees in Agro-Ecosystems

Environmental assessments with biotic indicators are carried out to detect changes or predict the potential effects of a given practice or stressor on a specific group ganisms. Indicators must respond to the practice or measure being addressed and the localities for which they must be valid (Osinski et al. 2003).[60]

Wild bees are well accepted biological indicators, especially for ecological assessment in open landscapes such as arable regions or grasslands (see Schwenninger 1994)[71] [38]Kevan 1999, Weber 1999, Plachter et al. (2002).[83] So far, however, the inclusion of bees in environmental risk assessment is restricted to honey bees and, in a few cases, to bumblebees (Kevan et al. 2008, Romeis et al. 2008). Particularly honey bees have often used as non-target organisms to test the impacts of pesticides (Thompson 2003).[78] Quite recently new rearing methods for honey bee larvae have been developed to test e.g. the effect of pesticides or the impact of GM crop pollen on bee larvae (Hendriksma et al. 2011a,b). Only few studies deal with the impact of pesticides on bees (e.g. Gretenkord (1997)[26], Ladurner et al. (2005)[46] Whitehorn et al. (2012)[88] but no agreed risk assessment procedures for them have yet been established. These laboratory methods are part of a standardized risk assessment, which examines toxicity as a direct effect of an agent to bees. The potential effects of a pesticide at the level of the bee community have almost remained unconsidered (Morandin and Winston 2005, Holzschuh et al. (2008).[81] Tuell and Isaacs (2010) tested a method to compare pest management programs for their potential effect on wild bee communities. So far, however, we lack studies dealing with the indirect effects of environmental stressors on wild bee communities as well as descriptions of standardized methods for long-term studies to measure changes of wild bee communities in agricultural landscapes.

Standardized Ecological Assessment With Wild Bees

The use of wild bees in ecological assessment makes it necessary to develop stand ardized methods and a precise study design. It must be considered that studies have to produce verifiable and reproducible results. For environmental assessment projects with wild bees we recommend the combination of transect walks and pan traps (see section 2). This method is likely to deliver reliable results on the diversity of wild bee communities and the abundance of wild bee species. Particularly for long-term studies, our methodical design provides the following advantages:

- Live observation of bees in combination with net collecting reduces the percentage of killed individuals to < 30% of the recorded specimens (Schwenninger unpubl). This approach helps to minimize negative effects of monitoring on bee populations.
- The combination of transect walks and pan traps (bowls) provides high sample coverage. Since pan traps are used only temporarily, their impact on size and composition of bee populations is negligible.

The suggested method can operate efficiently in a wide range of ecological monitoring schemes in agricultural ecosystems. We point out that comprehensive methodical, biological and taxonomical expertise is required. We disagree with Westphal et al. (2008),[84] who, for long term monitoring schemes, recommend pantraps as the most suitable method for surveyors with different levels of bee taxonomic expertise. It should be considered that long term exposed pan traps are very effective at trapping bees and could negatively influence local bee populations. For this reason we reject long term passive sampling methods.

Conclusion

There is still a current need for biotic indicators to evaluate the impacts of land use change as well as the effects of agr environmental schemes on biodiversity (Buchs (2003)[12] Osinski et al. 2003, Bergschmidt 2004, COM 2006). Wild bees are approved bio indicators (Kevan 1999).[38] However, guidelines for standardised ecological assessment by using wild bees are currently missing. Our described methodical design can be applied in many contexts (e.g. impact of land use changes, evaluating ecosystem services, climate change, invasive bee diseases) to different agricultural

landscapes in Europe. In future the application of wild bees as biodiversity indicators might also be ofrelevance in monitoring potential effects of GM crops (see Meier and Hilbeck 2005).

The interpretation of changes in the diversity of wild bee communities and the abundance of certain wild bee species is made difficult because of a lack of former studies in many regions in Germany and Central Europe. Moreover changes of wild bee communities could be attributed e.g. to annual fluctuations in population size, arising from natural variation in population growth, or to the species' dispersal ability (see Murray et al. 2009).[58] Therefore we strongly recommend standardized base-line studies with replications in different agricultural landscapes. Initially the base line should be documented through a three-year field study. Subsequent studies should be repeated every five years to record the status quo and the changes in biodiversity of wild bee communities. Longterm base line monitoring will provide data that allow distinguishing between the natural population dynamics of wild bee species and effects attributed to environmental changes. An assessment using biotic indicators such as wild bees requires crucial ecological and taxonomic qualifications. Therefore we encourage the establishment of courses to qualify collaborators for our suggested monitoring schemes with wild bees.

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Studies On Seed Collection, Seed Germination and Ex-Situ Conservation of Some Plants From Gautala Sanctuary

Anil A. Kshirsagar[1], P.K. Sanghai[2]

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Abstract

The Gautala sanctury is reserve for plant biodiversity and wild life situated in Marathwada region dist. Aurangabad Tq. Kannad. The forest is rich in wild and local varieties of plants which are becoming rare due to natural and human activities. It is important to conserve and study the plant biodiversity. In this regard it is decided to collect the seeds of various plants and study the germination percentage and various aspects. The present paper deals with the studies on collection, ex-situ conservation and seed germination of five plants. Seeds were collected during respective seasons and germinated. The overall percentages of seed germination in between 50-80 percent are noted.

Keywords: Ex-situ conservation; Seed collection; Germination with its percentage etc.

Introduction

Gautala sanctuary of Kannad is protected area of Maharashtra state, India. It lies between Satmala and Ajanta hill ranges of Western Ghats. It is wildlife sanctuary established in the year 1986 in an existing reserve forest area. India is rich with flowering plants and having mega diversity in country. Every flowering plant bears seed. Seeds are one of the important parts of the plant. Seed germination is a critical stage of life cycle of plants. According to Z.huang et al.[7], R.H.Yang et al.[5] Seed germination is the critical stage for survival of species. It is rather extraordinary to think that one small seed can grow a tree that will live for many years reaching to several meters in heights. But seed are delicate part to maintain their potential to sprouting for that purpose the seed must be collected, stored and treated properly. There are more than thousands of seed banks in the world for ex-situ conservation of plant diversity. According to D.J. Merritt and Dixon[2] seed bank collections of wild species will play an increasingly important role in restoration, regeneration of plant species. Ex-situ conservation utilizes proven methods by which seeds are available when need to be used for species recovery. Currently the importance of timing of seed collections to increase seed longevity in storage. Seed germination characteristics to maximize the use of seeds in recovery. The seed and seedlings establishment is one of the initial stages of plant development. The germination process is a specific for each species, depending on different factors like seed maturity, physical

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conditions, harvesting methods Kandari et al.[3],V. Gupta[6]. The aim of this present study is to focus on seed collection, ex-situ conservation and seed germination of some plants.

Materials and methods

The mature seeds of five different plants were collected at suitable conditions and stored in plastic containers by using preservative, before storing to check the germination percentage of seeds. The seed of Albizia lebbeck, Aloe vera, Bauhinia variegata, Cassia fistula and Butea monosperma was used for experiments. The seed germination percentage can be calculated by following formula.

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total seeds taken}} \times 100$$

Observations

1. **Albizia lebbeck (L).Benth. (Fig. 1a)**

   Common Name: Shirish

   Flowering: March-May

   Family: Fabaceae

   Fruiting: July-Feburary

   Habitat: It is commonly found in dry deciduous forest, also planted along the roadsides. It is tall tree; the leaves are bipinnate, 15cm long with 1-4 pairs of pinnate. Flowers are white yellow with numerous stamens and very much fragrant.

   Seed Morphology: The seeds of this plant are macroscopic 0.7–1.0 x 0.5–0.7 x 0.1–0.4cm, oblong ovate, compressed, seed coat is hard, and seed surface is smooth.(Fig. 1b)

   Seed Germination: Seed germinate within 7-15 days and germination capacity is about 80%.(Fig. 1c)

2. **Aloe vera (L) Burm.F. (Fig. 2a)**

   Common Name: Korpad

   Flowering: December-February

   Family: Asphodelaceae

   Fruiting: March-May

   Habitat: It is a stem less plant, very short stem, leaves are thick and fleshy. It is cultivated for its commercial products such as skin lotions, beverages, ointments, cosmetics and also used for digestive juice; gel is also made from succulent leaves etc. Flowers are yellow to orange fruit dehiscent capsule.

   Seed Morphology: The seeds of this plant are macroscopic 5-9 x 2.0-5cm. Seed coat is thin transparent papery, surface of leaf is scaly. (Fig. 2b)

   Seed germination: Seed germination takes place within 12-20 days and germination percentage is 18-25%. (Fig. 2c)

3. **Bauhinia variegata L. (Fig. 3a)**

   Common Name: Kanchan, Kanchana

   Flowering: November-January

   Family: Fabaceae

   Fruiting: January-March

   Habitat: It is a small medium sized tree growing up to 15 meters in height. The bark is light brownish to grey. The flowers are variegated to white or purple coloured very much attractive arising terminally or auxiliary in position. Mostly auxiliary racemes or corymbs, fruits are dry dehiscent, pods 10-16 seeded, oblong hard and flat in structure.

   Seed Morphology: The seeds of plant are macroscopic 1.1–1.5 x 1.0 x 1.4 x 0.1–0.2 cm. flat,orbicular,slightly pointed tip ,seed coat is hard, oval slit, surface is smooth to rough with pointed tip.(Fig. 3b)

   Seed germination: Seed germination is near about 80% and takes 25-35 days. (Fig. 3c)

4. **Cassia fistula L. (Fig. 4a)**

   Common Name: Bahava (Golden shower tree)

   Flowering: April-June

   Family: Fabaceae

   Fruiting: May-January

   Habitat: It is one of medium sized deciduous or semi-deciduous tree. Near about 10-15 meter tall. The branches are spread to form an open crown. The stem is with bark which is smooth and slender and rough when it is old. Flowers are yellow in colour raceme; it is also called as golden shower tree due to its attractive flowers. Fruit is indehiscent legume nearly about 30-80 seeded elongated pendulous. The legume has pungent odor and containing several seeds. The tree has hard durable wood.

Seed Morphology: Seeds are macroscopic 7.1 -9.2 x 6.0-7.1 x 2.3-3.4 mm, obcordate compressed usually flat, seed coat is hard, surface is glossy sometimes scalariform striations along margins or all over surface etc. (Fig. 4b)

Seed germination: seed germination period is 50-7 %.(Fig. 4c)

5. **Butea monosperma L. (Fig. 5a)**

Common Name: Palas or palathi

Flowering: December-March

Family: Fabaceae

Fruiting: April May

Habitat: It is small sized tree growing up to 15 meter. The leaves are pinnate with 8-15 cm long petiole, it is trifoliate. The flowers are bright orange red produced in raceme. Fruits are indehiscent pods. Single seeded, oblong or broadly linear.

Seed Morphology: Seeds are macroscopic 2.4 -3.4 x 2.0-2.5 x 0.1-0.3 cm, reniform, compressed, seed coat is thin, vertically veined, rugose, surface glossy. (Fig. 5b)

Seed germination: seed germination takes about 25-65 days and percentage of germination is above 70%. (Fig. 5c)

Result and discussions

The present study concerning with collection and conservation of seeds with seed germination percentage of five different plants were evaluated. The seeds are collected from natural habitats the ample seeds of plants were taken and count the percentage of seed germination A. Raghav et al.[1] and M. Thirupathi et al.[4]

Table 1: Showing germination percentage of the plant species.

Sr. No.	Name of plant	Germination percentage	Days require
1	Albizia lebbeck (L)Benth.	80 %	7-15
2	Aloe vera (L)Burm .F.	18-25%	12-20
3	Bauhinia variegata L.	80 %	25-35
4	Cassia fistula L.	50-70 %	50-75
5	Butea monosperma L.	70 %	25-65

In above studied plants the seed germination percentage is near bout 70-80% recorded. The lowest percentage noted in Aloe vera and highest percentage of seed germination noted in Albizia lebbeck and Bauhinia variegata.

Fig. 1a: Albizia lebbeck, **Fig. 1b:** Seeds of A. lebbeck, **Fig. 1c**: Germination of A. lebbeck

Fig. 2a: Aloe Vera, **Fig. 2b)** Seeds of A. Vera, **Fig. 2c)** Germination A. Vera

Fig. 3a: Bauhinia variegata, **Fig. 3b)** Seeds of B.variegata, **Fig. 3c)** Germination B.variegata

Fig. 4a: Cassia fistula, **Fig. 4b)** Seeds of C. fistula, **Fig. 4c)** Germination C. fistula

Fig. 5a: Butea monosperma, **Fig. 5b)** Seeds of B. monosperma, **Fig. 5c)** Germination B. monosperma

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