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Seasonal dynamics of arthropods in Lucerne (*Medicago sativa* L.) and nutritional link to rodent foraging

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Abstract

Lucerne (*Medicago sativa* L.) is a highly nutritive crop with more vitamins and minerals used as a fodder legume since it is available throughout the year. In the Coimbatore district farmers prefer lucerne as a fodder crop because of its quality, year-round production and seed yield fetches a high price. The monitoring study on lucerne crop pests, natural enemies and pollinators was conducted at Tamil Nadu Agricultural University, Coimbatore for three seasons from October 2021 to June 2024. The major sucking pests observed in Lucerne were aphids (*Aphis craccivora* Koch) and stink bugs (*Nezara virudula*, (L.)) and the natural enemy observed was coccinellid predators (*Cheilomenes sexmaculata* (Fabricius)). Natural enemies such as the Ladybird beetle (*Coccinella repanda* Thunberg) and Paper wasp (*Polistes exclamans* Vierick) were also documented as crucial in regulating these pest populations. The study also observed a diverse range of pollinators including the Alfalfa Leafcutter bee (*Megachile rotundata* (Fabricius)), various butterflies and several species of bees that are vital for effective pollination and enhanced crop quality. The population of aphids and stink bugs ranged from 0.00 to 1.47 and from 0.00 to 1.35, respectively, from April to May. In contrast, from November to February, the aphid population varied from 0.07 to 0.45, while the stink bug population ranged from 0.02 to 0.38. The maximum population of coccinellids was recorded at 3.16 in October 2023. The correlation of pest and predator population with weather parameters showed maximum and minimum temperatures favoured the population development while it was negatively correlated with relative humidity and rainfall. Profiling of root nutrients showed lucerne roots contain a significant amount of essential nutrients that meet the dietary requirements of mice.

Keywords

arthropods; ICP-MS; Lucerne; mice; root nutrient profile

Introduction

Lucerne, originating in Asia, has become a vital legume forage crop in many countries globally. It is suitable for hay and silage making and used as forage for sheep and cattle (1). Lucerne has become an economic forage crop for its nutritional quality, short crop period, high water use efficiency and fixing atmospheric nitrogen. Although lucerne produces fodder in a short period, various pests and natural enemies have an impact on its quality and yield. About a hundred insect pests are observed in Lucerne (2) and aphids (*A. craccivora*) are the most important pest due to the significant level of infestation and it increases the coumestrol content of Lucerne which shows the impact on the ovulation of grazing animals (drylandpastures.com). Stink bugs (*N. viridula*) are known to infest a wide

range of plant families, with a significant preference for legumes (3). The nutritional composition, plant architecture and availability of resources like moisture and sunlight contribute to this preference. The insect's ability to thrive on these plants can lead to significant crop damage, affecting yield and quality. In many crops, pesticide spray for other pests will also take care of stink bugs. In fodder crops, chemical pesticides are entirely restricted; hence, the stink bug population becomes significant. Any eco-friendly pest management approach starts with monitoring insect pests which is a core part of Integrated Pest Management and necessitate the study to monitor the sucking pests and natural enemies of lucerne crop in which pesticides are restricted.

Rodents, birds and ungulates are serious pests that damage field crops, vegetables, fruits and fodder crops in arid regions (4). In India, significant changes in agricultural practices over recent decades have contributed to an increasing rodent problem. In the present study, along with insect pests, mice have been observed in lucerne fields and the damage caused by these rodents is greater in lucerne compared to other crops in the same ecosystem. Hence, to investigate the factors that attract mice to lucerne crops, a study on root nutrient profiling was conducted using the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) technique to compare the nutrient content with the dietary needs of mice.

Materials and Methods

Field monitoring

The field monitoring was conducted at the Department of Forage Crops farm, Tamil Nadu Agricultural University, Coimbatore by Fixed plot survey during the period of *Rabi* 2021 - *Zaid* 2024. The observation was carried out in the field plots sown with RL 88 lucerne variety and ten plants were randomly selected in each plot and recorded the presence of pests, natural enemies and pollinators (if any). Aphids were counted from the 5 cm shoot tip of each plant; the number of stink bugs was recorded on a whole plant basis and coccinellid grubs and adults were also registered in each randomly selected plant. The average number of pests per plant was calculated and correlated with weather parameters using the SPSS software. Data on pest population and weather parameters were entered as variables and analyzed. The overall presence of pests, natural enemies and pollinators was observed during the study period and documented.

Root nutrient profiling using ICP-MS

The lucerne crop was found to be damaged by rodents and to examine the factors/nutritional elements responsible for mice attraction, root nutrient analysis was performed. Inductively Coupled Plasma-Mass Spectrometry [ICPMS], Thermo Scientific™ iCAP™ RQICP-MS is an analytical instrument used in this study to determine trace elements is equipped with micro mist borosilicate glass nebulizer; quartz cyclonic spray chamber; ICP torch, nickel sampler cone and skimmer cone, Quadrupole mass analyser and mass spectrometry detector. All the samples were analyzed in Kinetic Energy Discrimination (He KED) mode using pure He as the collision gas in the collision/reaction cell (CRC) under optimized auto-tune conditions of the equipment directly from Quality control with Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software.

Preparation of Calibration Standard solutions

Accurately 1 mL of mixed standard reference solution pipetted into a 100 mL volumetric flask diluted to the volume of 100 mL using HPLC grade water. This was taken as the stock solution used for the preparation of calibration standard solutions and stored under suitable conditions. Appropriate aliquots were taken and further diluted with 5 % nitric acid in HPLC grade water to give a series of calibration standard solutions with concentration ranges of 1.0, 20.0, 50.0 and 100.0 µg/L.

Preparation of sample solutions

Lucerne plants were unearthed meticulously to obtain an intact root system and were cleaned, dried and subjected to oven drying at 60ºC until the sample reached a constant weight. The dried sample was ground using a mortar and pestle to achieve a fine, homogeneous powder for accurate and uniform analysis. The homogenized powder is then subjected to a digestion process performed through a closed-vessel microwave digestion system - Multiwave GO (Anton Paar) with a multi-wave pro rotor, temperature and pressure sensor, provided with an auto pressure vent PTFE vessel. Wherein, 0.2 g of the sample was weighed in a microwave digestion vessel and 6 mL of nitric acid and 1 mL of hydrochloric acid to dissolve the sample completely. This step is critical to prepare the sample for analysis in the ICP-MS system. The vessel was placed in the fume hood for 15 min after which the digestion process was initiated.

The digestion was carried out in three steps with constant microwave power, the program was set to increase the temperature to 160 °C in 10 min and held at that for 5 min and cooled down the digester at room temperature and diluted the digested samples with HPLC grade water up to 50 mL. Three replicate samples were prepared for each sample for analysis.

After filtration, the sample was introduced into ICP-MS wherein, the aerosol was released into an ICP torch, where it was ionized at very high temperatures. The ions were then directed into the mass spectrometer, where they were separated based on their mass-to-charge ratio (m/z).

ICP-MS operating conditions

RF power (W) 1500	Nebuliser pump (rpm) 40
Plasma gas flow rate (L min-1) 14	Extract lens (V) 1.5
Auxiliary gas flow rate (L min-1) 0.8	Pirani pressure (mbar) 1.81
Carrier gas flow rate (L min-1) 1	Penning pressure (mbar) 3.05x10-7
Spray chamber T (°C) 2	Plasma exhaust (mbar) 0.45-0.56

Results and Discussion

Field survey and correlation with weather parameters

In the field survey study, the major pest recorded in the lucerne crop was cowpea aphid (*A. craccivora*) during the *Rabi* and *Zaid* seasons of the crop from October 2021 to March 2024. In the *Rabi* season of 2021-2022, the population of aphids ranged from 0.25 - 1.23/plant (Table 1). Aphid population was found to be maximum during May 2022 (1.23/plant) and minimum during

December 2021 (0.20/plant). In the *Rabi* season of 2022-2023, the aphid population was minimum in November 2022 and February 2023 whereas the maximum population was found in May 2023. In the *Rabi* season of 2023-2024, the aphid population was first observed in October 2023 after which the population was drastically reduced. Correlation studies revealed that both maximum and minimum temperatures favoured the aphid population, whereas relative humidity and rainfall showed a negative impact (Table 2). Studies by (5) reported that the aphid population initiated during the third week of February slowly increased in the subsequent weeks and reached its maximum (10.22/10 cm twig) level during the third week of March. *A. craccivora* is a minor pest in Lucerne but occurred in fifty host plants (6). Reports by (7) showed that *A. craccivora* is a major pest in Lucerne and its incidence was maximum from January to April which is in line with present findings. The occurrence of *A. craccivora* in Lucerne during March-May was registered by (8). The weather parameters *viz.,* temperature and relative humidity had an impact on the aphid population (9).

The heteropteran pest in lucerne recorded in this study was stink bug *N. virudula* and the minimum population was during December 2021 (0.25/plant) and maximum during May 2022 (1.35/plant). In the *Rabi* season of 2022-2023, the minimum population of stink bugs were recorded in January 2023 (0.06/ plant), whereas the maximum population was observed in April 2023 (0.30/plant). In the *Rabi* season of 2023-2024, the stink bug population was first observed in October 2023 (0.02/plant), after which the population was drastically reduced. The stink bug population was shot up by maximum and minimum temperatures and negatively correlated with rainfall and relative humidity (Table 2). Soybean is the primary host for *N. viridula* and is also reported in other legumes (3). The growth and development of *N. viridula* in soybeans occurred during summer and shelters in other crops during other seasons. In many crops, spraying for significant pests will reduce the population of *N. viridula*. Otherwise, it is a pest of more crops and not restricted by global boundaries. Apart from abiotic factors, the host plant also plays a vital role in the population dynamics of *N. viridula* (3). The preference of *N. viridula* towards legume crops is also reported by (10).

Coccinellids (*Cheilomenes sexmaculata*) were found to have occurred throughout the study period and were high in October 2023 (Table 1). Even though the overall population was low, it was significant during the summer months. *C. sexmaculata* is active year-round in many regions of India and has multiple generations. Its key characteristics include wide geographical distribution, diverse host range, adaptability to habitats, pesticide resistance, enhanced searching ability and strong larval feeding capacity (11). The most preferred host for *C. sexmaculata* is *A. craccivora* (12) which is most abundant in legumes. *C. sexmaculata* is a potential predator of soft-bodied insects and is registered as more influential on *A. craccivora* in legumes (13). It was also reported in Lucerne in the summer months by (14). Since *C. sexmaculata* has a diverse host range, it occurs in crops sequentially within the same ecosystem and lucerne may act as a shelter crop for this potential predator. Tritrophic interaction relies on environmental conditions and population dynamics of *A. craccivora* and *C. sexmaculata* altered

Table 1. Seasonal occurrence of the insect pests and natural enemies in Lucerne

Date & Month	Aphids (Mean No. of aphids/ plant)	Stink bug (Mean No. of nymphs and adults/plant)	Natural enemies
			Coccinellid beetle (Mean no. of grubs and adult/plant)
October, 2021	0.44	0.26	0.16
November, 2021	0.25	0.38	0.13
December, 2021	0.20	0.25	0.08
January, 2022	0.24	0.26	0.08
February, 2022	0.25	0.38	0.10
March, 2022	0.75	0.75	0.20
April, 2022	1.18	1.28	0.24
May, 2022	1.23	1.35	0.28
October, 2022	0.54	0.10	0.42
November, 2022	0.42	0.12	0.45
December, 2022	0.45	0.12	0.37
January, 2023	0.46	0.06	0.30
February, 2023	0.42	0.17	0.35
March, 2023	1.07	0.12	0.45
April, 2023	1.42	0.30	0.35
May, 2023	1.47	0.22	0.40
June, 2023	0.47	0.18	0.45
October, 2023	0.20	0.02	3.16
November, 2023	0.07	0.02	2.00
December, 2023	0.07	0.07	0.85
January, 2024	0.00	0.08	1.22
February, 2024	0.00	0.02	0.90
March, 2024	0.75	0.35	1.12
April, 2024	0.00	0.00	0.15
May, 2024	0.00	0.00	0.00
June, 2024	0.00	0.00	0.20

Table 2. Correlation of Pests and Natural Enemies in Lucerne with weather parameters

	2021 -2022			2022-2023			2023-2024		
	Aphids	**Stink bugs**	**Coccinellids**	**Aphids**	**Stink bugs**	**Coccinellids**	**Aphids**	**Stink bugs**	**Coccinellids**
Maximum Temperature	.704**	.703**	.542**	0.640**	0.436**	0.070**	0.252	0.097	-0.295
Minimum Temperature	.760**	.650**	.497**	0.498**	0.411*	0.291*	0.083	-0.142	-0.286
RH (7-22 hrs)	-.279	-.374*	-.238	-0.105	-0.132	0.117	-0.362*	-0.279	-0.303
RH (14-22 hrs)	-.132	-.204	-.079	-0.111	-0.082	0.273	0.403*	-0.308	-0.101
Rainfall	-.225	-.255	-.052	-0.091	0.335*	0.087*	-0.102	-0.107	-0.257

*Correlation is significant at the 0.05 level (2 - tailed)

** Correlation is significant at the 0.01 level (2- tailed).

by temperature and rainfall (15).

The correlation of aphid, stink bug and coccinellid populations with weather parameters was given in Table 2.

Documentation of other insect pests, predators and pollinators

Apart from key pests, the other insect pests *viz.,* Pumpkin beetle *Aulacophora abdominalis*, Cow bug *Oxyrachis tarandus,* Mottled stink bug *Rhaphigaster nebulosa,* Tobacco caterpillar *Spodoptera litura,* Pod bug *Riptortus pedestris* were also documented in the Lucerne field of which bugs are dominant one. Coccinellid beetles are documented as dominant predators. The honey bees, which are predominant pollinators in every cropping system, in Lucerne also explored more. The other pollinators that visited Lucerne flower are the Alfalfa Leaf cutter bee *Megachile rotundata,* blue butterfly *Lampides boeticus,* Common grass yellow butterfly *Eurema hecabe,* Swallow tail butterfly *Pachliopta polydorus,* Monarch butterfly *Danaus chrysippus,* Sweat bees *Agapostemon texanus,* Blue banded bee *Amegilla cingulata* and Buckeye Butterfly *Junonia lemonias* (Fig. 1).

Root nutrient profiling and mice dietary requirement

Trace elements in lucerne roots were identified using the ICP-MS instrument (Table 3). Lucerne roots contain significant amounts of essential nutrients that meet or exceed the dietary requirements of mice. The amount of calcium in Lucerne roots (7.54 g/lit) is higher than the required amount for mice (5 g/kg of diet) (Table 4). Lucerne roots provide a substantial amount of potassium (11.52 g/lit), far exceeding the mice's dietary requirement (2 g/kg). In addition, Sodium (Na), Magnesium (Mg), Phosphorus (P) and Iron (Fe) levels in Lucerne roots also meet or exceed the levels required by mice. Micronutrients such as molybdenum (Mo), copper (Cu), manganese (Mn) and zinc (Zn) are present in Lucerne roots, albeit in smaller quantities. However, the presence of these trace elements can help to fulfill the rodents' nutritional needs for these micronutrients. Lucerne roots provide a well-rounded source of both macro and micronutrients that can sustain mice. This nutrient richness might explain why rodents are attracted to Lucerne root as they provide a natural and comprehensive source of their nutritional needs.

The comprehensive nutrient profile of lucerne roots likely explains their attractiveness to rodents. Research has indicated that rodents tend to select food sources rich in essential nutrients (16). The availability of both macro and micronutrients in lucerne makes it a preferred food source. Lucerne was reported as the most preferred crop by ungulates followed by rodents in the Jodhpur district of Rajasthan (4).

Table 3. Trace elements documented from lucerne root sample

Elements	Lucerne Root (in ppm)
7 Li (Lithium)	2.057
9 Be (Beryllium)	0.213
11B (Boron)	28.432
23Na (Sodium)	7835.866
24Mg (Magnesium)	4900.578
27Al (Aluminium)	3143.001
31P (Phosphorus)	3500.164
39K (Potassium)	11535.009
44Ca (Calcium)	7544.482
48 Ti (Titanium)	55.624
51V (Vanadium)	4.136
52Cr (Chromium)	13.509
55Mn (Manganese)	40.373
57Fe (Iron)	512.819
59Co (Cobalt)	0.994
60Ni (Nickel)	7.007
63Cu (Copper)	4.507
66Zn (Zinc)	2.873
75As (Arsenic)	0.127
95Mo (Molybdenum)	0.387
107Ag (Silver)	0.006
111Cd (Cadmium)	0.017
118 Sn (Tin)	0.296
121Sb (Antimony)	Nil
133Cs (Cesium)	0.033
137Ba (Barium)	12.447
202Hg (Mercury)	Nil
205Tl (Tellurium)	0.007
208 Pb (Lead)	1.932
60Ni (Nickel)	7.007

Table 4. Comparison of trace elements in lucerne root and amount of elements required for mice diet

Nutrient requirement of Mice	Amount per kg of diet (in g)	Amount in Lucerne root (g/lit)
Calcium	5	7.54
Copper	0.06	0.004
Zinc	0.1	0.002
Potassium	2	11.52
Molybdenum	0.00015	0.0003
Sodium	0.5	7.83
Magnesium	0.5	4.94
Phosphorous	3	3.50
Manganese	0.10	0.04
Iron	0.35	0.512

Pumpkin beetle

Cow bug

Mottled stink bug

Tobacco caterpillar

Pod bug

Lady bird beetle

Paper wasp

Alfalfa leaf cutter bee

Blue butterfly

Common grass yellow butterfly

Swallow tail butterfly

Monarch butterfly

Carpenter bee

Sweat bee

Honey bee

Blue banded bee

Buck eye butterfly

Fig. 1. Common pests, predators and pollinators documented in lucerne.

Conclusion

Lucerne crop is available throughout the year and not receive pesticide applications so it can harbour more natural enemies. Lucerne ecosystem harbours various natural enemies and it can be recommended for intercrop or border crops to conserve natural enemies and sustainable pest control. Continuous monitoring is essential for registering the role of pests in qualitative and quantitative loss and identifying natural enemies for eco-friendly pest management. The nutrient richness of lucerne roots makes them an attractive food source for rodents. With essential macronutrients exceeding the dietary requirements of mice and the presence of vital micronutrients, lucerne roots serve as a well-rounded nutritional option. This nutritional profile not only supports the health of mice but also explains their attraction to lucerne, suggesting that such plants play a crucial role in their foraging behavior and dietary preferences.

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Authors' contributions

KP conceived and designed the study, contributed to data collection, supervised the research and contributed to manuscript writing and editing. JNP contributed to data analysis, manuscript writing and editing. SL conducted laboratory analysis and RP assisted in the experimental design and manuscript preparation. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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