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A STUDY ON POPULATION DYNAMICS, DNA BARCODING, AND STEM FLY MANAGEMENT BLACK GRAM IS INFESTED WITH MELANAGROMYZA SOJAE

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Abstract

Cytochrome oxidase subunit I (COXI) molecular characterization seems to lend credence to the specimen's identification as a stem fly, M. sojae, feeding on black gramme. This study is the first to report M. sojae as a pest of black gramme in India. The pest population in the black grame crop planted in the first and second weeks of March was low: 28.38 and 29.73% infestation, 8.92 and 9.45% tunnelling, 0.22 and 0.26 larvae/plant, and 0.27 and 0.27 pupae/plant. Infestation was low (22.69%) and tunnelling was low (3.85%) in the black gramme crop planted in the third week of July for the kharif harvest. In compared to crops planted in August, larval and pupal counts were lower in crops planted in the third and fourth weeks of July. The crop that was planted in the second week of March yielded the most seeds (597 kg/ha), followed closely by the crop planted in the first week of March (564 kg/ha). Seed and haulm yields were both highest for crops planted in the third week of July during the kharif season (806 and 429 kg/ha, respectively).

Keywords: *Population Dynamics, DNA Barcoding, Fly Management, Black Gram.*

1. Introduction

India, Pakistan, and Bangladesh are just a few of the nations that cultivate black gramme (Vigna mungo) for its useful nutritional and economic qualities. Melanagromyzasojae, a stem fly, is a prominent pest of black gramme because it feeds on the plant's stem and leaves, causing large output losses. Studies of population dynamics are crucial for comprehending the activities and interactions of pests with their surroundings. Population dynamics research into Melanagromyzasojae may reveal the influence of variables such host plant density, climate, and natural enemies.[1]

DNA barcoding is a method of species identification that makes use of very few, short genetic markers. Using this method, Melanagromyzasojae may be positively identified and

differentiated from other closely related species. DNA barcoding may be used to learn more about Melanagromyzasojae populations, including their genetic makeup and how they are distributed geographically. Managing stem flies in black gramme crops may be difficult due to the high cost and potential environmental damage associated with using chemical pesticides. Melanagromyzasojae populations may be reduced with the use of integrated pest management practises such the introduction of natural enemies and the cultivation of resistant cultivars. The creation of efficient control measures may be aided by knowledge of the population dynamics and genetic diversity of the pest.[2]

Protein, vitamins, and minerals may all be found in good quantities in pulses. The United Nations has designated 2016 as the International Year of Pulses in recognition of the numerous positive effects that pulses may have on human health. Increasing the production of pulses is crucial not only to ensure that people have enough protein in their diets, but also to ensure that people are aware of the importance of pulses in ensuring both nutritional food security and environmental sustainability. Since pulses are leguminous, they may be included into a broad variety of cropping systems, making them an essential part of maintaining agricultural productivity. The tap root structure of these plants increases soil porosity while also enhancing soil fertility and physical wellness. Black gramme root nodules have been used as a cure for aching bones, dropsy, and cephalgia due to their narcotic and diuretic properties. Pulses are a class of legumes; they are agricultural plants in the family Leguminosae that are grown for their edible seeds. Pulses are exclusively dry-grain-harvested legumes.[3]

Historically, black gramme cultivation has been concentrated in tropical and subtropical regions during the kharif growing season. India, Pakistan, Sri Lanka, Burma, and a few Southeast Asian nations are major producers. In 2018-2019, India harvested 30,59,990 tonnes of black gramme from a total area of 56,02,470 ha, yielding 546 kg/ha productivity. In 2018-2019, black gramme output in Gujarat was 73560 tonnes, with a productivity of 669 kg/ha over an area of 1,09,960 hectares. Sabarkantha, Panchmahal, Dahod, Vadodara, Mehsana, and Bharuch are the most important districts in Gujarat for the production of black gramme. The districts of Rajkot, Surendranagar, and Junagadh also engage in little cultivation.[4-5]

The 100-gram serving of black gramme comprises 25 percent protein, 56 percent carbs, 18 percent dietary fibre, 1 percent fat, 3 percent minerals, 4 percent lysine, 1 percent methionine, 0 percent tryptophan, 0 milligrammes calcium, 345 milligrammes phosphorus, and 8

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milligrammes iron. Pulses, both whole and split, are often eaten as a nutritious addition to a grain-based diet. Dal-chawal (pulse-rice) and dal-roti (pulse-wheat bread) are staples in the typical Indian diet. Because of the complimentary link between the important amino acids, such as arginine, leucine, lysine, isoleucine, valine, phenylalanine, etc., the protein quality in the diet is much increased when pulses are taken with cereals. In addition to being a necessary component of human and animal diets.[6-7]

2. Literature review

Galinskaya, T. V. &Shatalkin, A. I. (2020)established a statistically significant negative association between Madurasiaobscurella Jacoby's damage to green gramme (V. radiata) in Killikulam, Tamil Nadu, between kharif and rabi, and maximum temperature, lowest temperature, sunlight hours, and wind velocity. Researchers discovered that higher humidity levels led to more damage. Wind speed was positively correlated with agromyzid damage, whereas high and low temperatures, relative humidity, and sunshine hours all had a negative effect.. The kharif season saw a preponderance of M. obscurella, whereas the rabi season saw an abundance of O. phaseoli.[8]

Ambenagare, R. M. &Takankhar, V. G. (2019) Researchers in Ganj Basoda, Madhya Pradesh, looked at how weather affected the seasonal occurrence of insect-pests of soybean and found that the infestation of the stem fly (M. sojae) started on 28 DAG in the first week of August, 2010 with 10% plant infection and 0.30% stem tunnelling. The highest rate of plant infestation (73.3%) and stem tunnelling (48.50%) occurred during the third week of September, 2010. There was a population reduction in response to any rise in precipitation, minimum temperature, or relative humidity.[9]

Chiang, H. S. and Norris, D. M. (2018)M. sojae, a fly that feeds on soybeans, was shown to reduce crop output by 30–50% in Parbhani, Maharashtra. Plants infected with M. sojae ranged from the 27th to the 38th SMW (12.70 to 27.20%) in 2010–2011, and from the 30th to the 40th SMW (10.90 to 25.70%) in 2011–2012. The lowest temperature in 2010-2011 was positively and strongly linked with the percent infestation of M. sojae (r=0.584*), according to the data on correlation coefficient. There was no statistically significant correlation between the percent of M. sojae infestation and factors such rainfall, wet days, maximum temperature, morning and evening RH. During 2011–12, the data on correlation coefficient revealed that the percent infestation of M. sojae was favourably correlated with evening

relative humidity (0.674*), but not with rainfall, wet days, maximum and lowest temperature, or morning RH.[10]

Dey, D. &Trimohan (2017)maximum temperature was shown to be the most significant determinant in the presence of the stem fly, O. phaseoli, in black gramme in Tirupati, Andhra Pradesh. The second two weeks of February and the second two weeks of March had average highs of 36°C to 38°C, ideal conditions for the stem fly. At its peak, the infection rate at this time was a staggering one hundred percent. During the peak occurrence time, morning relative humidity was measured at 68-76% and evening relative humidity at 22-32%.[11]

Dodia, D. A. & Tikka, S. B. S. (2016) Initial efforts to amplify the COX1 mitochondrial gene DNA barcoding region using the universal LCO1490 HCO2198 primer combination had a low success rate, especially in samples collected before to 2001. Forty-five tephritid fruit fly specimens, all from collections made between 1961 and 2005, were selected. Attempts to generate a DNA barcode using the folmer primers on these specimens were fruitless. None of the samples produced a PCR result, and seven of them could not be sequenced. Only a small number of freshly gathered examples were used to evaluate and enhance the whole collection. These freshly obtained samples correctly amplified all of the provided markers. Newly developed primers (L1440d & H2123d) for the whole Tephritid DNA barcode (dubbed the Teph658 marker) recovered an amplicon for 30% of the samples when used in lieu of the folmer primers. After 1999, 50% of samples were successfully amplified with the new primers, but only 23% were using the folmerprimers. [12]

3. Methodology

The management of the black gram-infesting stem fly, M. sojae, requires attention to population dynamics, DNA barcoding, and pesticide use. Sowing timings, intercropping impacts, varietal screening, seed treatments, and pesticide use in the soil are some of the research materials and methodologies used to analyze population growth, DNA barcoding, and management.

3.1 Population dynamics of stem fly

The GU 1 (Gujarat Urad 1) type of black gramme was used in a study of the stem fly population dynamics. The crop was cultivated in the farm of the B. A. College of Agriculture, A. U. Anand, during the summer and kharif seasons of 2017 and 2019, respectively, for this purpose.

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3.1.1 Details of Experiment

Location	:	Entomologyfarm,BACA,AAU,Anand		
Cropandvariety	:	Blackgram,GujaratUrad1		
Plotsize	:	20 x 10 m		
Spacing	:	45 x 10 cm		
SeasonandYear	:	Summer(2017 & 2019) and*Kharif*(2017& 2019)		
Fertilizerdose(N:P:K)	:	20-40-00kg/ha		
Date ofsowing	:	Year Season	2017	2019
		Summer	22/03/2017	17/03/2019
		Kharif	14/08/2017	10/08/2019
DateofHarvesting	:	Year Season	2017	2019
		Summer	14/06/2017	11/06/2019
		Kharif	20/11/2017	16/11/2019

3.1.2 Methodology

The plant population dynamics were studied by cultivating the crop in 20 x 10 m plots spaced at 45 x 10 cm. There were no procedures taken to safeguard the plants in the allotment. There were six equal sections of land on the site. Ten plants were studied from each of the four areas. Ten plants were picked at random from each plot and transported to the lab to check for stem fly infection.

The instantaneous effect of weather parameters on stem fly population fluctuations was analysed by correlating data of abiotic parameters such as bright sunshine (BSS), rainfall (RF), wind speed (WS), maximum and minimum temperature, morning, evening relative humidity, morning, evening vapour pressure, and evaporation. Department of Agricultural Meteorology, B. A. College of Agriculture, Anand Agricultural University, Anand, India, weekly data of climate variables collected between 2017 and 2019 were used.

3.2 DNA barcoding of stem fly, Melanagromyzasojae (Zehntner)

These samples were frozen at -20 degrees Celsius until DNA extraction. QIAGEN DNeasy blood and tissue kit (Qiagen) was used to extract DNA from all samples in accordance with the manufacturer's instructions.

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Agarose gel electrophoresis and a Nanodrop ND-1000 spectrophotometer version 3.5 were used to examine the DNA sample for both quality and quantity.

With a 25 ul reaction volume, 50 ng of starting DNA, and 2X PCRMastermix (EmeraldAmp® GT, Takara), the COXI gene was amplified by polymerase chain reaction (PCR). The PCRMastermix included 0.05 U/ul Taq DNA polymerase in reaction buffer. Conditions for PCR amplification of the human COXI gene

3.2.1 Sequence analysis

In order to modify and assemble the acquired COXI gene nucleotide sequences, clone management was used. Species were successfully identified by utilizing a BLAST search at NCBI on the acquired consensus sequences. MEGA6's CLUSTALW programme was used to align the COXI sequences produced in this work. MEGA6 was used to infer evolutionary connections. For this phylogenetic tree, we employed the Neighbor-joining technique. This DNA barcoding research made use of equipment from the Anand Agricultural University's AICRP on Biological Control of Crop Pests.

4. Results

Very little is known about M. sojae in Gujarat, including its seasonal occurrence, DNA barcoding, impact of sowing period, screening of black gramme genotypes/cultivars, impact of intercropping, or bio-efficacy of seed treatment or granular insecticides. Therefore, the current inquiries were conducted in summer 2017 and 2019 and kharif 2017 and 2019.

4.1 Stem Fly, M. sojae, Population Dynamics in Black Gramme

The current study on the stem fly, M. sojae, and population dynamics was conducted at the Entomology farm, B. A. College of Agriculture, Anand Agricultural University, Anand, India, during the summers of 2017 and 2019, and the kharif seasons.

Based on the computed t-test (2.33*), which demonstrated a statistically significant difference in infestation between pooled over-year data of summer and kharif (53.33%) seasons (Table 4.1), we may conclude that there is a substantial variation in infestation between 2017 and 2019. However, the level of infection was negligible in each season of each year, and each year of each season.

Table 4.1: Black gramme stem fly (M. sojae) infection varies with the seasons and years

Year Season	2017	2019	t-test	Pooledover years
Summer	56.47	57.40	NS	56.94
Kharif	49.31	50.12	NS	49.72
t-test	NS	NS	-	**2.33***
Pooledoverseasons	52.89	53.76	NS	53.33

4.1.1Meteorological variables and black gramme M. sojae infestation matrix

There is never a completely constant population of insect pests in nature. Many abiotic elements, such as temperature, rainfall, humidity, etc., influence the increase and decrease of any organism's population density. A simple connection was calculated between the mean values of numerous meteorological factors and the mean infestation of M. sojae on black gramme over the course of three days.

Infestation and weather conditions were shown to have a positive correlation with minimum temperature (MinT) and morning vapour pressure (MoVP) ('r' value of 0.509** and 0.522**, respectively). The evaporative humidity pressure (EvVP) in the evening (0.440*) also correlated positively. There was a positive correlation between BSS, RF, WS, MaxT, MoRH, and EvRH (morning relative humidity, afternoon relative humidity, and evening relative humidity), and a negative correlation between evaporation and the other variables.

A statistical examination of how M. sojae infestations are affected by weather conditions is shown in Table 4.2. Very strong positive correlations ('r' values of 0.769** and 0.510**, respectively) were found between infestation and minimum temperature (MinT) and evening vapour pressure (EvVP). There found a favourable correlation between MoVP (morning vapour pressure) and PV (0.495*). The abiotic factors we examined (rainfall, wind speed, maximum temperature, evening relative humidity, and evaporation) were positively correlated, whereas the biotic factors we assessed (bright sunshine hours, morning relative humidity, and evaporation) were negatively correlated.

Table 4.2: Black gramme infestation by M. sojae in summer and the correlation (r) between meteorological variables

	Correlationco-efficient(r)

WeatherParameters	Summer,2017	Summer,2019
BrightSunshineHours(BSS),hr/day	0.278	-0.184
WindSpeed(WS),km/hr	0.145	0.346
MaximumTemperature(MaxT),^{o}C	0.213	0.074
MinimumTemperature(MinT),^{o}C	0.509**	0.769**
MorningRelativeHumidity(MoRH), %	0.172	-0.210
EveningRelativeHumidity(EvRH),%	0.301	0.388
MorningVapourPressure (MoVP), mmofHg	0.522**	0.495*
EveningVapourPressure (EvVP),mmofHg	0.440*	0.510**
Evaporation(EP),mm	-0.124	0.161

Table 4.3 displays the results of a statistical analysis of the relationship between M. sojae infestation and climatic variables. Both the number of bright sunlight hours (BSS) and the maximum temperature (MinT) showed significantly significant positive correlations with the number of infestations. Rainfall (RF) was significantly correlated negatively with evaporation (EP) (-0.458*), whereas evaporation was significantly correlated positively with RF (0.466*). Morning vapour pressure (MoVP) was negatively correlated with the other abiotic variables studied (wind speed (WS), minimum temperature (MinT), morning relative humidity (MoRH), evening relative humidity (EvRH), and morning vapour pressure (MoVP), and evening vapour pressure (EvVP) were positively correlated).

Table 4.3: The r-value for the association between kharif-season climatic conditions and M. sojae infection in black gramme

WeatherParameters	Correlationco-efficient(r)	
	***Kharif*,2017**	***Kharif*,2019**
BrightSunshineHours(BSS),hr/day	0.519**	0.085
Rainfall(RF),mm	-0.458*	0.169
WindSpeed(WS),km/hr	-0.329	-0.430*
MaximumTemperature(MaxT),^{o}C	0.655**	0.050
MinimumTemperature(MinT),^{o}C	-0.075	0.038
MorningRelativeHumidity(MoRH), %	-0.448	0.151
EveningRelativeHumidity(EvRH),%	-0.358	0.006
MorningVapourPressure (MoVP), mmofHg	-0.129	0.086

EveningVapourPressure (EvVP),mmofHg	0.001	0.055
Evaporation(EP),mm	0.466*	-0.017

Table 4.3 displays the results of a statistical analysis of the relationship between M. sojae infestation and climatic variables. Wind speed (WS) was shown to have a strong negative link with pest infestation (-0.430*), as calculated by the correlation coefficient between infestation and abiotic parameters. The investigation of the association between stem fly infestation and other meteorological factors did not find any linear relationship between the two. There was a positive correlation between the number of bright sunshine hours (BSS), the amount of rainfall (RF), the highest temperature (MaxT), the lowest temperature (MinT), the morning relative humidity (MoRH), the evening relative humidity (EvRH), the morning vapour pressure (MoVP), and the evening vapour pressure (EvVP), and the evaporation rate (EP).

4.2 DNA Barcoding of Stem Fly

4.2.1 DNA extraction and quantification

To learn more about species authentication and DNA barcoding, a molecular characterisation of stem flies that have been feeding on black gramme crop was conducted. The Nanodrop spectrophotometer was used to determine the concentration of the isolated DNA. DNA was present at a quantity of 30–110 ng/l. High-quality, protein- and RNA-free genomic DNA was utilised for further study.

4.2.2 Polymerase chain reaction (PCR) of COXI gene

Genomic DNA was extracted from the sample, and the COXI (Cytochrome c oxidase subunit 1) gene was amplified using polymerase chain reaction (PCR). Amplifying the COXI gene required the use of both a forward primer (LCO 1490, 5'-GGTCAACAAATCATAAAGATATTGG-3') and a reverse primer (HCO 2198, 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). The COXI gene fragments were amplified using the usual PCR procedure described in the methodology section, and then examined using 1.5% agarose gel electrophoresis. During the study, a single amplicon of the COXI gene, measuring 650 bp in length, was observed. (Fig. 4.1).

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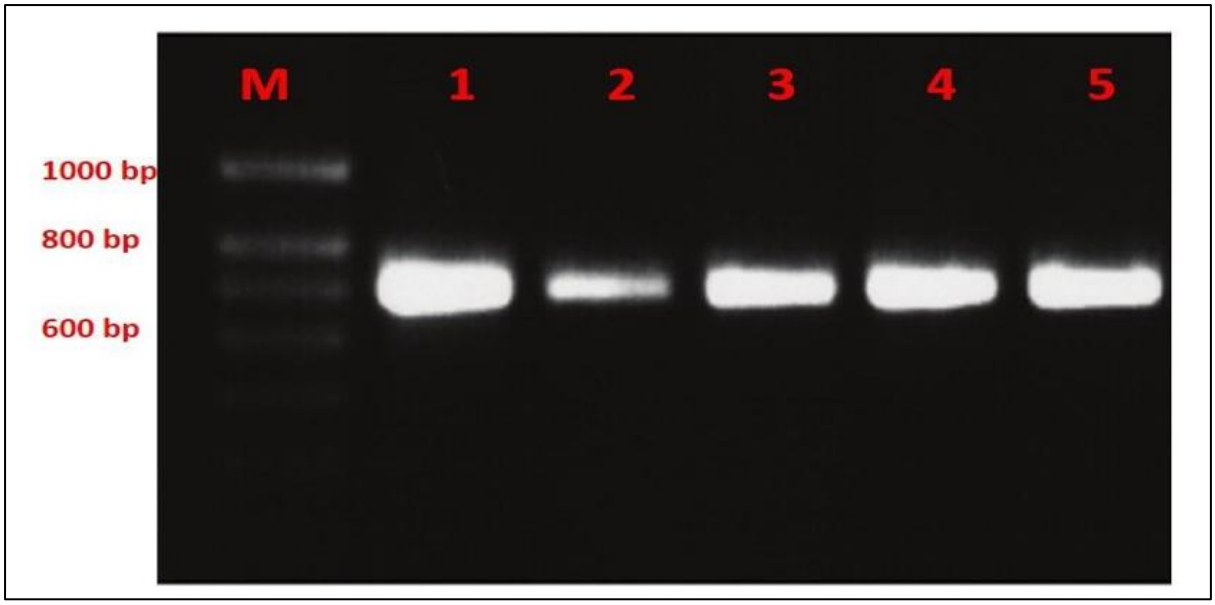

Fig. 4.1: Melanagromyzasojae's AAU-Anand voucher material was utilised for PCR amplification of the 650-bp COX1 gene.

4.2.3 Sequence analysis and retrieval from NCBI database

Using NCBI-BLAST, we found that the consensus sequence of the COXI gene in the stem fly specimen was similar to the sequences of many other dipteran insect COXI genes in the NCBI database. Melanagromyzasojae was the identification provided by the ICAR-National Bureau of Agricultural Insect Resources (ICAR-NBAIR), Bengaluru, India, using morphological keys. Additionally, the NCBIBLAST programme was used to authenticate the species by restricting the query coverage to Melanagromyza sp. The percentage of similarity between three Melanogromyza sp. specimens from the NCBI database with accession numbers KR661203, KR655154, and KR645071 ranged from 87.84 to 87.99%. Additionally, Melanagromyzaobtusa was the most common species of Melanagromyza, with similarities between 87.12 and 88.04%.

5. Conclusion

The black gram-infesting stem fly, M. sojae, has its identification confirmed by molecular characterization, and a DNA barcode was generated. Analysis of the cytochrome oxidase subunit I (COXI) gene led to the identification of the genus as Melanagromyza sp. Similarity analyses between the AAU-black gramme specimen and the Melanagromyza sp. KR661203, KR655154, and KR645071 in the NCBI database ranged from 87.84 to 87.99%. Three intercropping scenarios were compared to determine the impact of black gramme on stem fly occurrence: black gramme with maize (3:2 and 5:1), black gramme with sorghum (3:2 and

5:1), and black gramme as a solitary crop. Black gramme crops with maize (2:1) or sorghum (3:2) as intercrops had summertime average stem fly infestations of 26.96 and 28.13 percent, respectively.

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