

# RECENT ADVANCES IN AGRICULTURAL RESEARCH

Editors

Burak SALTUK

Osman GÖKDOĞAN



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Agricultural Sciences

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## **Recent Advances in Agricultural Research**

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## FOREWORD

Agricultural production is important in today's world and new studies are needed on the subject. Further the offering micro and macro level contribution to economy, agricultural production is also important for human nutrition, and it is one of the most indispensable industries of the world. It is clear that agricultural products are strategically important. The significance of agriculture, food and health industries was particularly recognized during the pandemic.

This book is a collection of various studies and consists of 5 chapters. This book will clearly become a reference for academicians and students in related departments. We would like to thank the scientists from different universities who contributed to the conveying of the book to its readers and everyone who contributed to the publication of the book.

We believe that current studies conducted by the editors and the editorial board will contribute to the future studies to be conducted.

Sincerely Yours,

**Assoc. Prof. Dr. Burak SALTUK**

**Assoc. Prof. Dr. Osman GÖKDOĞAN**



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# CHAPTER I

## DETERMINATION OF AFLATOXINS M<sub>1</sub>, AFLATOXINS B<sub>1</sub> AND TOTAL AFLATOXIN BY ELISA IN CIVIL CHEESE SAMPLES AND THEIR MONTHLY DISTRIBUTION

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### **1. Introduction**

Cheese according to the Turkish Food Codex Cheese Communiqué; “By coagulating the raw material with a suitable coagulant and separating the whey from the curd or it is obtained by coagulating the permeate of milk after separation, with different hardness and fat content, salted with brine or dry salting or without salting, with or without starter cultures, boiled or not boiled curd, with or without flavorings, produced according to the technique, consumed before or after ripening, dairy products with variety specific characteristics” was defined as (Anonymous, 2015). Because of its high B2, vitamin A, protein and calcium contents, cheese plays a crucial role in meeting our daily nutritional needs (Arslan & Kadı, 2021). In our country, there is more than one type of cheese production technique due to geographical differences, cultural diversity and different breeds of animals raised according to the regions. In general, cheese is made by acid treatment, yeast (rennet) treatment or heat treatment. Civilian cheese is a special cheese that is produced without using any of these methods in its production (Arslaner & Salık, 2020).

Civil cheese is a kind of cheese produced in Eastern Anatolia, particularly Erzurum, and known and consumed throughout Turkey. A geographical indication is a sign that clearly shows the unique characteristics of the product, fully identifies with its origin and indicates the main source of the product

(Saygılı, Demirci & Samav, 2020). The cheese, which is also known by other names such as “çeçil” or “iplik, tel”, was granted a geographical mark by the Turkish Patent Institute on 06.02.2009 and was restricted to a region in which Erzurum and its districts are located. This cheese is a fat-free cheese. Therefore, its popularity is growing, as it is a delicious alternative for consumers who want to avoid dairy products high in fat and cholesterol. The cheese can be classified in the group of pasta filata cheeses because it is produced by blanching and kneading and has its own flexible, non-porous and fibrous structure. The yield of civil cheese depends on various factors and varies between %7-%10. Civil cheese can be consumed as a brine or, after maturing, in barrels or plastic containers together with brydza and curd made from whey, or it can be consumed fresh. It is usually ripened without the use of starter cultures. The molted civil cheese is mostly preferred due to its special taste and aroma, but it has the risk of mycotoxicosis as a result of molting (Arslaner, & Salık, 2020; Erdogan, Gurses, Turkoglu, & Sert, 2001; Gurkan, Yilmaztekin, Cakmakci, & Hayaloglu, 2018). Mycotoxins are toxic metabolic products produced by fungi under appropriate conditions (temperature, humidity, etc.). Mycotoxins are transmitted to humans in 2 different ways. It is transmitted through the consumption of the animal’s meat, milk and eggs as a result of the ingestion of food or feed containing the toxin or the consumption of feed containing mycotoxins (Deveci & Sezgin, 2016).

Aflatoxin (AF), ochratoxin A, fumonisin, trichothecene and zearalenone are considered as the major mycotoxins. AFs are among the most dangerous mycotoxins. These toxic compounds are the most potent carcinogenic and mutagenic secondary metabolites produced specifically by *Aspergillus flavus* and *Aspergillus*. AFs are produced by *Aspergillus ochraceoroseus*, *Aspergillus toxicarius*, *Aspergillus arachidicola*, *Aspergillus minisclerotigenes* involved in *Aspergillus* genu and by *Emericella astellata*, *Emericella venezuelensis* and *Emericella olivicola* involved in *Emericella* genu and also by *Rhizopus spp.* *Penicillium spp.* do not produce AF, but the species of this genus do produce AF under certain conditions. However, this level of importance in the ranking may differ between countries (Eseceli, Atan & Günşen, 2019). AF consists of four main fractions, namely B1, B2, G1 and G2, which are expressed as AFB1> AFG1> AFB2> AFG2 according to the toxicity power they produce (Deligöz & Bilge, 2017). AFM1 and AFM2 have been detected in the milk of animals feeding on AFB1 and AFB2 and contaminated feed as a result of hydroxylation of these toxins. (De Roma, Rossini, Ritieni, Gallo & Esposito, 2017; Mehenktaş, 2019;

Ozkan & Onmaz, 2019). AFB1 has been classified as a first-order carcinogen and AFM1 as a second-order carcinogen by the International Agency for Research on Cancer (IARC) (Bukari, Kwofie & Adeboye, 2020; Rushing & Selim, 2019). In order to determine the status of civil cheese according to the standards in terms of AFM1, it was agreed that 1 kg of civil cheese is produced from 100 kg of milk and the maximum limit for AFM1 was set at 0.5 µg/kg. In the previous regulations of the Turkish Food Codex, a maximum limit of 5.0 µg/kg AFB1 and 10.0 µg/kg total AF (B1+B2+G1+G2) was set for foods listed as “other foods (at-risk foods likely to be found)”. In the research carried out on the presence of AF in cheese, especially in civil cheese, it has focused on AFM1. When cheese is made from milk contaminated with AFM1, the toxin enters the curd.

Therefore, AFM1 levels vary depending on various factors (processing techniques, differences in milk quality, etc.) (Mehenktaş, 2019). It was reported that in the previous researches on civil cheeses regarding the presence of AF, no observable amounts of AFM1, AFB1, AFB2, AFG1 and AFG2 were detected. In further researches, the presence of AFM1 in civil cheese samples was reported at different rates. It was found that in %14.44 of braided cheese, which is a similar type of civil cheese, the AFM1 rate was higher than the permissible rate (250 ng/kg) specified by the Turkish Food Codex (Anonymous, 2015).. However, in another research, no sample was found to exceed the limit of 500 ng/kg in civil cheese samples. There is no study on the determination of AFM1, AFB1 and total AF concentrations in civil cheeses in relation to the monthly distribution in Erzurum/Turkey. Various methods (ELISA, HPLC and TLC) have been used to determine mycotoxins in food and feed. The ELISA for AFM1, which has been included in the official collection of test methods by the Federal Ministry of Health (Germany), is rapid, reliable and cost-effective. In this study, it is aimed to investigate the presence of AFM1, AFB1 and total AF, which may be hazardous to human health, in samples of civil cheeses produced in Erzurum and to determine the monthly distribution.

## **2. Materials and Methods**

### ***2.1. Cheese Sample Collection***

A total of 120 civil cheese samples were collected randomly (10 samples per month) from different regional markets in Erzurum from January to December. The samples were transported to the laboratory in a plastic bag in the refrigerator. Samples were kept at 2-4°C until analysis.

## ***2.2. Determination of AFM1, AFB1 and Total AF by ELISA Method***

AFM1, AFB1 and total AF concentrations of the samples were analyzed by ELISA methods using the RIDASCREEN® Aflatoxin M1, (Art. No: R1101), RIDASCREEN® Aflatoxin B1, (Art. No: R1201) and RIDASCREEN® Aflatoxin Total, (Art. No: R4701) ELISA kits according to described procedure by R-Biopharm GmbH (Darmstadt, Germany). Samples were analyzed using the Rida®Soft Win programme provided by R-Biopharm AG.

## ***2.3. Statistical Analysis***

SPSS 13 software (SPSS Inc., Chicago, USA) was used for statistical analysis of the obtained data. First, Kolmogorov-Smirnov and Shapiro-Wilk tests were performed to check the distribution of the data. Kruskal-Wallis analysis of variance (ANOVA) was then used to measure statistical differences in AFM1, AFB1 and total AF concentrations between months, while Differences in AFM1, AFB1 and total AF concentrations between months were analyzed using the Mann-Whitney U test. Statistically significant was defined as a P value <0.05.

## **3. Results and Discussion**

People living in Erzurum/Turkey prefer Civil cheese for local consumption every month of the year. AFs are considered to be a major health risk associated with the cheese. In the study, the concentrations of AFM1, AFB2 and total AF were determined by ELISA method in samples of 120 civil cheeses collected from different regional markets in Erzurum from January to December, and the results obtained are shown in **Table 1**.

**Table 1.** Concentrations of AFM<sub>1</sub>, AFB<sub>1</sub>, total AF in civil cheese samples by month and multiple comparisons.

Month	AFM <sub>1</sub> (µg/kg)	AFB <sub>1</sub> (µg/kg)	Total AF (µg/kg)
January	0.071±0.012 <sup>b</sup>	0.177±0.023 <sup>bcde</sup>	0.083±0.007 <sup>d</sup>
February	0.069±0.009 <sup>b</sup>	0.188±0.024 <sup>bc</sup>	0.084±0.009 <sup>d</sup>
March	0.064±0.009 <sup>bc</sup>	0.221±0.081 <sup>b</sup>	0.083±0.011 <sup>d</sup>
April	0.067±0.015 <sup>bc</sup>	0.141±0.066 <sup>e</sup>	0.088±0.008 <sup>d</sup>
May	0.068±0.009 <sup>b</sup>	0.174±0.061 <sup>cde</sup>	0.088±0.009 <sup>d</sup>
June	0.064±0.011 <sup>bc</sup>	0.162±0.025 <sup>de</sup>	0.162±0.039 <sup>c</sup>
July	0.070±0.014 <sup>bc</sup>	0.172±0.050 <sup>bcde</sup>	0.160±0.066 <sup>c</sup>
August	0.059±0.008 <sup>c</sup>	0.151±0.060 <sup>cde</sup>	0.175±0.041 <sup>c</sup>
September	1.160±0.099 <sup>a</sup>	0.440±0.326 <sup>bc</sup>	0.771±0.878 <sup>bc</sup>
October	1.205±0.966 <sup>a</sup>	0.316±0.242 <sup>bc</sup>	0.412±0.223 <sup>b</sup>
November	1.004±0.687 <sup>a</sup>	0.881±0.748 <sup>abcd</sup>	0.875±0.696 <sup>b</sup>
December	1.060±0.909 <sup>a</sup>	0.996±0.487 <sup>a</sup>	1.510±0.929 <sup>a</sup>
Mean	0.413±0.644	0.335±0.394	0.374±0.595

**Notes:** a, b, c, d : Means in the same line with the same letter are not significantly different ( $p>0,05$ )

According to the months, the AFM<sub>1</sub>, AFB<sub>2</sub> and total AF concentrations of the samples showed a statistically standard distribution as a result of the Kolmogorov-Smirnov and Shapiro-Wilk tests and it was found that the month factor has a significant effect on the AFM<sub>1</sub>, AFB<sub>1</sub> and Total AF concentrations of the samples ( $p<0.05$ ). The mean AFM<sub>1</sub> concentration of the cheese samples was found to be 0.413±0.644 µg/kg and it was determined that the lowest AFM<sub>1</sub> concentration was in August (0.059±0.008 µg/kg) and the highest AFM<sub>1</sub> concentration was in September (1.205±0.966 µg/kg). The AFM<sub>1</sub> concentrations of the months of March, April, June, July, August and September, October, November and December were found to be statistically indifferent ( $p>0.05$ ). When the civil cheese was evaluated according to the value (breakpoint: 0.5 µg/kg) based on the European Union regulations and the Turkish Food Codex (Anonymous, 2015)., none of the civil cheese samples analysed before September were found higher than the established value. However, 100 % of the samples analyzed in September and 70% of the samples analyzed in October, November and December were found to be higher than the established value.

Özgören & Kemal Seçkin (2016) stated that no AFM1 was detected in civil cheese samples exceeding the legal limit, while Aksoy & Sezer (2019) stated that some cheese samples exceeded the legal limit at different rates.

Erkan, Demir & Öksüztepe (2018) examined 100 samples of Şavak tulum cheese and stated that all samples contained AFM1 and exceeded the limits of the Turkish Food Codex (Anonymous, 2015). Mamedova, Atasever & Aliyev (2020), worked with a total of 110 cheese samples and as obtained results, while AFM1 above 5 ng/kg was detected in 49 (44.55%) of the samples, AFM1 was not detected in 61 (55.46%) samples and the AFM1 concentration of positive samples ranged from 5.19 to 16.42 ng/kg, with an average of  $8.00 \pm 2.43$  ng/kg. Considering these values, the mean values are quite low and the positive samples are compatible with the literature.

In the study conducted by Erkan, Vural & Güran (2019), Diyarbakır knitted cheese samples were analyzed for AFM1 between May and December 2013 and the mean AFM1 concentration of the samples was determined to be 167.58 ng/kg. In addition, the number of samples exceeding the limit set by the European Commission for AFM1 is 32 (17.39%). In the study achieved by Altun, Temamoğulları, Atasever & Demirci, M. (2017), the AFM1 concentrations of 130 cheese samples obtained from different markets in Diyarbakır, Erzurum, Mardin, and Şanlıurfa provinces were determined and AFM1 positive results were detected in all samples. In addition, it was found that 16.92% of the cheese samples examined were above the legal limit specified in the Turkish Food Codex (Anonymous, 2015).

In a study conducted to determine the rate of AFM1 in milk compared to the seasons, it was also stated that the AFM1 level in milk was higher in winter compared to other seasons, and they said that it was noted that the seasonal distribution of AFM1 concentration could be a result of the type of feed, production and storage conditions. Furthermore, it was stated that AF production was effective during the pre-harvest period (De Roma, Rossini, Ritieni, Gallo & Esposito, 2017).

In our study, the mean AFB1 concentrations of the civil cheese samples were found to be as  $0.335 \pm 0.394$  µg/kg, and the lowest AFB1 concentration was in April, ( $0.141 \pm 0.066$  µg/kg) and the highest AFB1 concentration was in December ( $0.996 \pm 0.487$  µg/kg). The AFB1 concentration in January was found to be statistically different from the concentration obtained in other months of the year, and the AFB1 concentration in December was found to be statistically different only from the concentration in November ( $p > 0.05$ ).

The mean total AF concentration of the samples was found to be  $0.374 \pm 0.595$   $\mu\text{g}/\text{kg}$ , with the lowest total AF concentration in January ( $0.083 \pm 0.007$   $\mu\text{g}/\text{kg}$ ) and March ( $0.083 \pm 0.011$   $\mu\text{g}/\text{kg}$ ) and the highest total AF concentration in December ( $1.510 \pm 0.929$   $\mu\text{g}/\text{kg}$ ). While the AF concentrations in January, February, March, April and May were found to be statically indifferent between each other ( $p > 0.05$ ), the total AF concentration in December was found to be different from all other months ( $p < 0.05$ ) (Table 1). The European Union regulations (European Commission (EC), 2010), the Turkish Food Codex (Anonymous, 2015), the Joint Expert Committee on Food Additives (Herrman, & Walker, 1999) have not set a limit for the tolerable intake level because the level of AF in food and its exposure in humans cannot be accurately determined. Instead, it is emphasised that countries should take measures to keep the intake of AF in the diet at the lowest level to minimise the potential risks and limit the consumption of foods containing AF to levels that cannot be reduced (Madalı & Ayaz, 2017). According to the Turkish Food Codex Contaminants Regulation (Anonymous, 2015), the maximum limit of AFM<sub>1</sub> in cheese varieties is 0.50  $\mu\text{g}/\text{kg}$  (Yeşil, Hatipoğlu, Yıldız, Vural & Erkan, 2019). In the limited number of studies, it was reported that AFB<sub>1</sub> and total aflatoxin were not found in civil cheese samples. It has been reported that there is a risk of mycotoxicosis in some cheese samples ripened with mould (Erdogan, Gurses, Turkoglu & Sert, 2001). In fact, the presence of AFB<sub>1</sub> and the total aflatoxin concentration at different levels in all cheese samples proves that there is a risk. In our result, the AFB<sub>1</sub> and total AF concentrations were above the levels specified in this Regulation. The research found that the months of September, October, November and December were the highest risk months for AFM<sub>1</sub>. It was felt that further research was needed, particularly into pre-harvest contamination. As far as AFB<sub>1</sub> and total aflatoxin are concerned, it can be said that the errors made, particularly in the production, packaging and storage processes, increase the available toxin rate in civil cheese that is ripened by molting, and thus have a serious potential of being a risk to public health. The fact that there is no maximum AFM<sub>1</sub>, AFB<sub>1</sub> and total aflatoxin concentration for the final product in the European Union regulations and the Turkish Food Codex (Anonymous, 2015) prevents the routine investigation of these toxins. Maximum limits for AFM<sub>1</sub>, especially in relation to the final product, AFB<sub>1</sub> for cheese matured by molting and total aflatoxin must be established in European Union regulations and the Turkish Food Codex (Anonymous, 2015).

#### 4. Conclusion

The present study is the first report showing the determination of AFM1, AFB1 and Total AF concentrations in civil cheese according to monthly distribution, in Erzurum/Turkey. It shows that continuous monitoring of AFM1, AFB1 and Total AF concentrations in civil cheese commonly consumed in Erzurum/Turkey should be done regularly because they also show a worrying situation.

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## CHAPTER II

# USE OF SNP CHIPS FOR GENOME-WIDE ASSOCIATION STUDIES IN LIVESTOCK

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### 1. Introduction

**S**elective breeding is a traditional method of farm animal husbandry. Moreover, it is regularly increasing knowledge about the genome. Determining genomic regions linked to complex traits is crucial for genomic selection and even economic profitability in livestock. Most molecular techniques positively affect breeding strategies by providing detailed genetic

maps of known quantitative trait loci (QTLs) associated with performance traits in farm animals (Fonseca et al., 2020; Singh et al., 2019).

GWAS, or whole-genome association study, is an effective technique to identify common genetic variants in individuals and their associations with a trait or traits. This method helps identify molecular markers suitable for disease and production traits, leading to efficient marker-assisted selection (MAS) or genomic selection (Freebern et al., 2020; Kirichenko et al., 2022; Pal, 2022; Sermyagin et al., 2020). Molecular markers are nucleotide sequences associated with a trait of interest, revealing polymorphisms at the DNA level. They are classified into two groups such as hybridisation-based or Polymerase Chain Reaction (PCR)-based markers (Adhikari et al., 2017; Salisu et al., 2018). An SNP marker is a single base change in a genome with a usual alternative of two possible nucleotides due to point mutations at a specific position (Liu & Cordes, 2004; Vignal et al., 2002). Various molecular methods have been used to detect SNPs. Among them, NGS has been commonly used for the cost-effective identification of genome-wide variation (Guan et al., 2022; Player et al., 2021; Roberts et al., 2022). SNP chips are DNA microarrays that test hundreds of thousands of site-specific mutations across the genome (LaFramboise, 2009). These chips have been widely used for analysing SNPs commonly found in the population (Lee et al., 2022). This chapter discusses GWAS, SNP chips, and their current usage in animal breeding and population genetics.

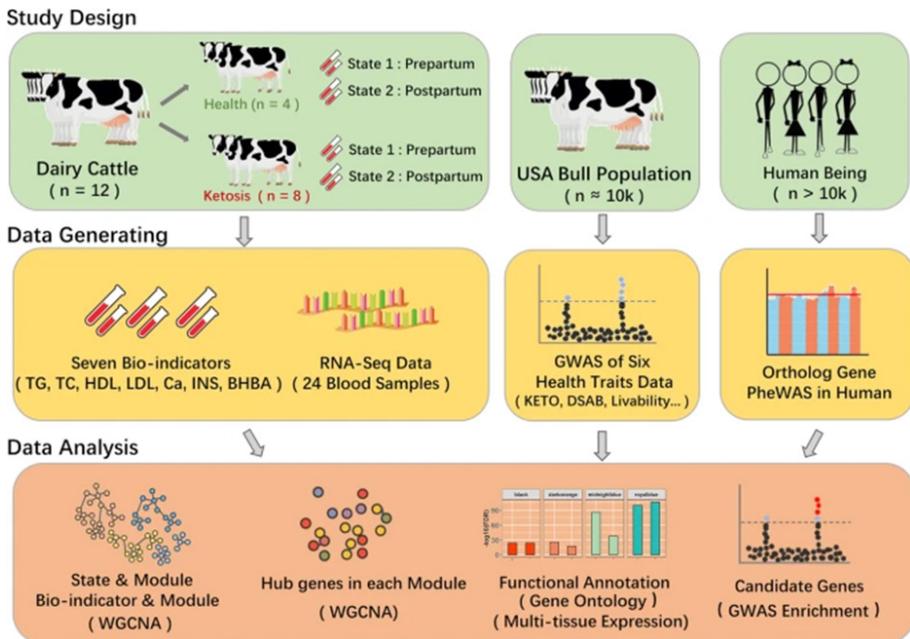
## 2. SNP

SNPs are described as the replacement of a single nucleotide with another nucleotide. However, the definition of SNP also includes the insertion or deletion of a single nucleotide. SNPs are detected in both coding and noncoding parts of the genome. They are one of the predominant marker types because of their richness in the genome. Advances in NGS technology and high-throughput genotyping methods have greatly facilitated broad SNP detection. Therefore, using positional cloning, MAS has effectively used SNPs to construct high-density genetic maps and isolate genes. Moreover, genome-wide SNPs aim to understand the genetic diversity, structure, and relationship among breeds in breeding and specialisation (Adhikari et al., 2017; Liu & Cordes, 2004; Nogueira et al., 2022).

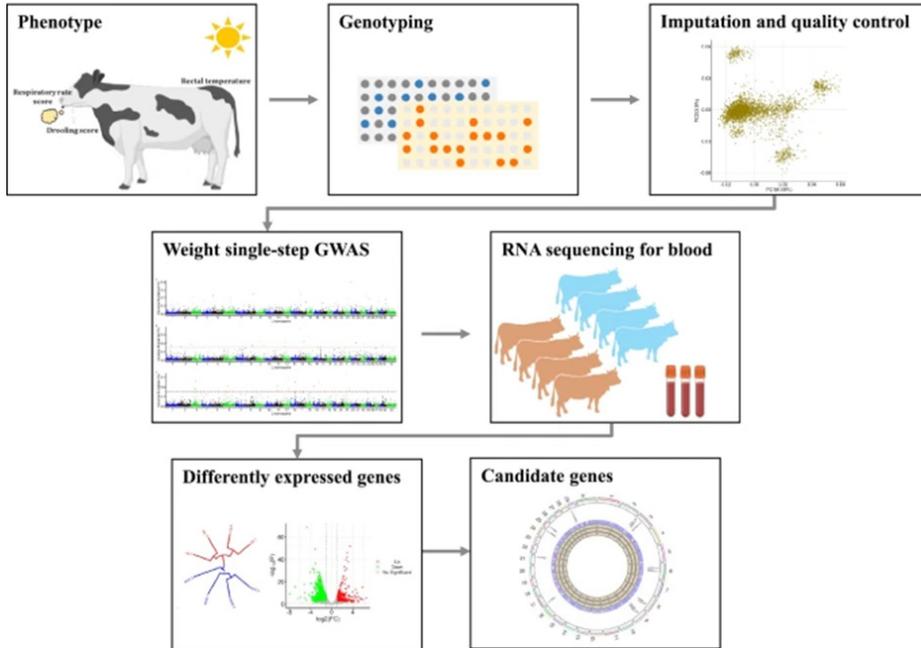
### 3. Genome-Wide Association Studies (GWAS)

GWAS is preferred for examining genome association with genetic diseases, resistance against pathogens, yield performances, and related phenotypes (Luo et al., 2022; Niciura et al., 2022; Wang et al., 2022; Zare et al., 2022) (Figures 1 and 2). Configuration of multiple tests is crucial to reduce wrong positive results in these studies. Many GWAS analyses are not effective for detecting SNP detection. Utilising new statistical analysis models on big datasets using high-performance computing (HPC) infrastructure is suggested in some recent literature (Cao et al., 2020).

Humans, plants, and animals include many highly quantitative phenotypes. Therefore, GWAS may fail to reveal the searched causal loci. Thus, improving phenotypic traits directly associated with genetic mechanisms may be the solution (Benjamin et al., 2012). This approach can increase the probability of detecting these loci by reducing the number of loci searched (Korte & Farlow, 2013).



**Figure 1.** GWAS and RNA sequencing for ketosis in cattle (Yan et al., 2020)



**Figure 2.** GWAS and RNA sequencing for heat stress in cattle (Luo et al., 2022)

DNA microarray chip technology is used for genotyping hundreds of thousands of common SNPs in GWAS. The significance level of GWAS is very high (corresponding to a very small p-value) due to multiple tests in which hundreds of thousands of SNPs are analysed. Most of these SNPs are commonly dispersed SNPs in a minor effect allele with a frequency greater than 5% within the population. GWAS findings require large-scale repetition and functional validation. Therefore, GWAS findings are usually assumed as hypothesis-generating studies (Smith et al., 2018).

Sufficiently practical statistical evaluation using a large sample size is not yet possible for whole genome studies. Thus, improving study efficiency using new statistical analysis methods is desirable. The development of new techniques has been accelerating in recent decades. Genetic mapping preliminary data providing information about the interrelationships of SNPs is called Linkage Disequilibrium (LD) (Slatkin, 2008) (**Figure 3**). New statistical models have often been devised by combining the LD relationships between SNP information from each well. The number of parameters in the LD matrix is  $n(n-1)/2$  ( $n$  is the number of analysed SNPs). Accordingly, all reliable models

include ambiguous LD information because it is unrealistic to obtain an accurate LD matrix estimate using a reasonable amount of samples in a genomic study (Cao et al., 2020).



**Figure 3.** Illumina BovineLD Genotyping BeadChip® (Anonymous, 2023)

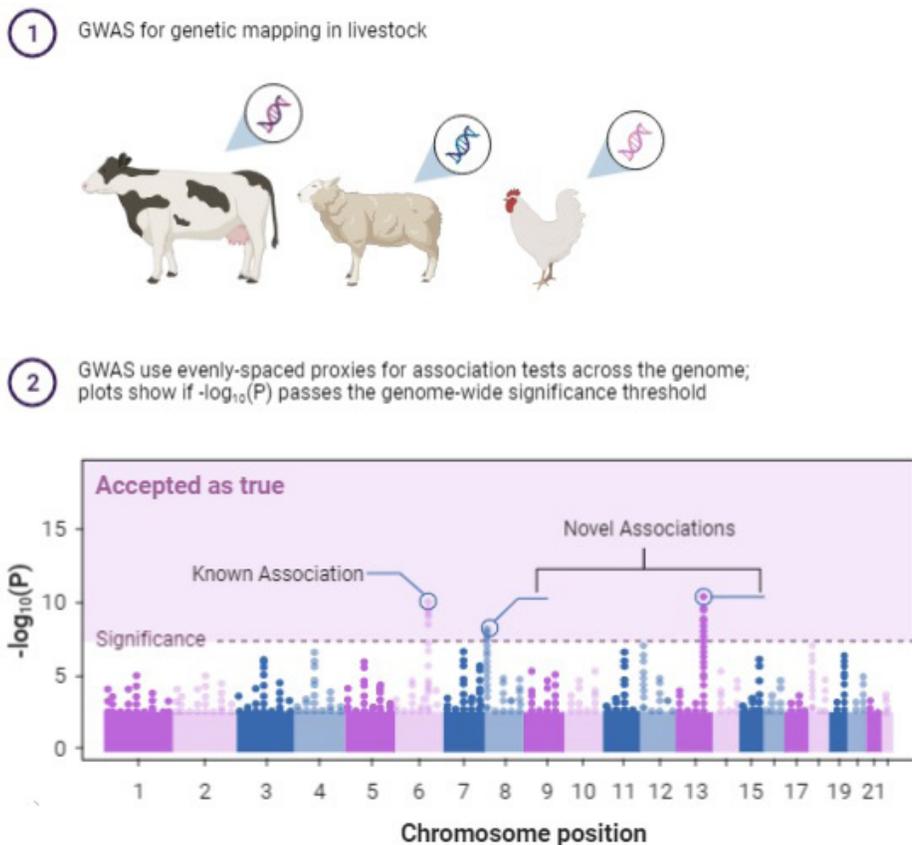
#### 4. SNP Chips in Livestock

SNP chips based on DNA microarray analyses were initially preferred to determine gene expression levels, but they have subsequently been used for the simultaneous scanning of SNP loci (Guo et al., 2023). In the DNA microarray method, probes with a known sequence are fixed on a microarray chip surface, a glass or nitrocellulose membrane. For analysis, a DNA or RNA representing expressed gene pool is isolated from different biological sources, amplified, and then labelled with a fluorescent dye. These probes are hybridised with possible complementary sequences (if any) in the solution. After washing to remove the target molecules that do not specifically bind to the probes on the chip surface, information about the sequences in the target solution is obtained with the help of a laser scanner. The emerging fluorescent display indicates the probes' positions where hybridisation successfully identifies targets in the analysed solution. The light intensity of each spot is considered a sign of the relative expression of a particular gene. Therefore, studying many regions simultaneously on a single chip is possible. DNA-based SNP examinations are used in molecular karyotyping, association mapping, admixture mapping,

identity by descent mapping, phenotype/genotype relationship correlations, and population genetic studies (Abdalla et al., 2022; Li et al., 2022; Mohammadi et al., 2022; Mukherjee & De, 2022; Pal, 2022).

SNP chips are classified as low density from 200 SNP panels to 35000 (35K) (Vallejo et al., 2018), medium density from 35K to 300K (Bejarano et al., 2018), high density from 300K to 750K, and ultra-high density above 750K (Ventura et al., 2019). Other studies evaluated SNP chips over 750K as high-density as appropriate (Khatkar et al., 2012; Mallikarjunappa et al., 2018).

Ongoing studies are carried out to prepare SNP chip panels with different resolutions for various farm animals. Some of the recent SNP chip development studies in cattle, sheep, goats, buffalo, chicken, turkey, and honey bees were reviewed in this chapter. Using of SNP chips on GWAS in livestock was schematised in **Figure 4**.



**Figure 4.** Using of SNP chips on GWAS in livestock (Kent et al., 2019)

#### **4.1. Cattle (*Bos taurus*)**

GWAS arrays in cattle have mainly focused on milk and beef yield. Moreover, SNP chips are also helpful for genetic resources and detecting local breeds. For this purpose, Khatkar et al. (2012) assessed the feasibility of SNP panels by using some low-density SNP panels such as Illumina; 3 and 7K, Affymetrix; 15 and 25K, and equally spaced subsets), medium-density SNP panels (Illumina 50K), and a high-density (Illumina 800K) SNP panels in dairy bovine genotypes. Although they found an allelic error rate of 0.79-4.78% in these panels, they reported that data from SNP chips with various density levels could be used to predict SNPs with a tremendous scale of correctness in population-based genotype implementations. They also indicated that low (3 and 7K) and medium (50K) density SNP chips have cost advantages in genomic predictions. However, medium-density chips make more accurate predictions but high (800K) density chips contribute less to accuracy. Thus, the researchers suggested that medium-density (50K) chips are reasonable options for accuracy and cost.

Another study was performed by Crispim et al. (2015). They discussed the parameters to describe beef bovine growth curves by using phenotypes within a GWAS model. Their analysed data were obtained from 1255 Brahman bovines at 6, 12, 15, 18, and 24 months. The GWAS analysis used different parameters, such as mature weight (A) and the maturity (K) ratio, from non-linear models as reciprocals of original body weights. While the genetic correlation between A and K was found as -0.84, animals with lower mature body weights were demonstrated to reach this weight at younger ages. As a result, 167 SNPs were associated with mature weight and 262 SNPs with maturity. Mature weight-related SNPs were reported as directly related to biological functions, such as; RAB28 for muscle development, BTG1 for myogenic induction, IL2 for fetal growth, and APEX2 for body weight. However, SNPs for K are indirectly associated with body weight and height, TMEM18 for daily gain and SMN1 for skeletal muscle growth. It has been noted that the candidate genes obtained from GWAS analysis can provide clues to causative mutations that support growth rate. Alam et al. (2021) examined three local and six foreign Taurine and Indicine cattle breeds. For this purpose, a phylogenetic tree was constructed using high-density (150 K) SNP chips (Affymetrix 150K SNP Axiom). Results showed the genetic uniqueness of Jeju Black cattle.

#### **4.2. Water Buffalo (*Bubalus Bubalis*)**

Based on morphological and behavioural criteria, water buffalos are identified as two distinct types (swamp and river). Iamartino et al. (2017) reported that the buffalo SNP chip (Axiom® Buffalo Genotyping Array 90 K) is suitable for analysing the genomes of both river and swamp buffalo breeds for genetic diversity studies. They reported that this SNP chip could also be used to analyse swamp buffalo. However, many loci were not informative, and they recommended the creation of a revised set of swamp buffalo-specific SNPs. In addition, Colli et al. (2018) identified genetic differences between two groups of water buffaloes (river and swamp buffalo) from different countries using the same SNP chip. Venturini et al. (2014) reported a group of SNPs that could affect water buffalo milk production and quality in another study.

Moreover, the cross-species transferability of SNPs by using 750K+ bovine SNP chips was reported by Iamartino et al. (2017). They designed a 90K Axiom® Buffalo Genotyping Array by sequencing the genomes of 73 water buffaloes from 4 different breeds and aligning them in bovine genomes. The researchers identified the genomic regions on chromosome 5 associated with milk production. In addition, they obtained a 70% rate of high quality and 24K SNPs.

In another study, Cesarani et al. (2021) aimed to test the effectiveness of genomic selection in milk and cheese (mozzarella) production parameters in 498 Italian water buffaloes. They suggested that yield could be increased using the BLUP method by using 90K SNP chips (Axiom® Buffalo Genotyping Array) in selection due to milk, fat, and protein yield analyses. They indicated no significant difference between the BLUP method and SNP genotyping in buffalo males, but there was an increase in all validation parameters in water buffalo females.

#### **4.3. Sheep (*Ovis Aries*)**

Sheep breeding is one of the leading livestock sectors with economic value, with meat, milk, and wool. A total of 14,845 sheep were investigated regarding growth, carcass, and meat quality traits using high-density SNP chips in New Zealand Terminal Sire breeds (Brito et al., 2017). The findings in the study showed that relatively accurate molecular breeding values could be predicted by progeny testing for a variety of traits in the early life of the lamb. Therefore, results give valuable information to short generation time and save costs. They suggested that this method would benefit complex and economically important

traits. However, these traits, such as carcass and meat quality, which can only be measured after slaughter, require a broad sample. Therefore, enhanced population size would be advantageous for making genomic predictions. It can be considered that the high density of SNP chips and the large population size are important factors for genomic predictions (Brito et al., 2017).

In another study, Pasandideh et al. (2020) used the Illumina Ovine 50K SNP chip to identify genomic regions associated with age at the first lambing and lambing interval in Baluchi sheep. GWAS analysis was performed in a fixed linear model with PLINK software. Two new SNPs significantly related to these characters were detected on chromosomes 10 and 15. These SNPs are found in the region encompassing the FAM155A and SYT13 genes of sheep, which are known to play a critical role in immune responses.

#### ***4.4. Goat (Capra Hircus)***

The goat is another economically important livestock with meat, milk, and hair. Unique selection and breeding strategies are prepared for each country, considering the conditions specific to that country. Thus, the first step is the genetic characterisation of populations. Visser et al. (2016) detected geographic and genetic variation in Angora (Cashmere) goat populations in Argentina, France and South Africa using a standardised 50K Goat SNP chip. The results showed that geographic isolations and unique breeding strategies led to genetic variations among populations. In addition, crossbreeding could increase specific populations' unfavourable traits, such as deficiency of reproductivity and low growth rates, while preserving superior mohair qualities. Jin et al. (2020) also determined the selection signals by comparing  $F_{ST}$  (fixation index) and XP-EHH (cross-population haplotype homozygosity test) indices in a non-cashmere breed and two cashmere breeds. They reported that WNT10A and CSN3 genes were potentially linked with cashmere properties. Furthermore, this information could be valuable for studying their genetic uniqueness and elucidating the mechanisms underlying cashmere traits in cashmere goats. The yield characteristics can be increased due to breeding programs applied in light of the data obtained using SNP chips.

#### ***4.5. Domestic Chicken (Gallus Gallus)***

Chicken and eggs have economic value in different industries. Additionally, chicken faeces are plausible organic plant fertilisers. Abdollahi-Arpanahi et al. (2014) aimed to distinguish marked additive genetic variations

for some quantitative traits in chickens through a complex genome-wide trait analysis approach, including minor allele frequency, individual chromosomes and marker density classes. A total of 1351 chickens were analysed, and they obtained 354,364 SNP genotypes from each chicken. These were analysed for body weight, breast muscle ultrasound, and hen house egg production. Variance component estimates indicated that commercially available SNP genotyping chips possessed large genetic variability in examined traits. The results showed that the contribution of SNPs for a marked additive genetic variance depends on the allele frequency spectrum. Marker density beyond 100K SNPs was not found to contribute to additive genetic variance for the analysed chicken samples. In another investigation, Liu et al. (2018) identified genomic variations associated with egg quality using 600K high-density SNP arrays in 1078 chicken populations at 72 and 80 weeks of age. Two candidate genes, MSX2 and DRD1, were mapped at the narrow critical region involved in embryonic and ovarian development associated with egg production. Furthermore, RHOA, SDF4, and TNFRSF4 genes were suggested as candidate genes for eggshell colour.

Liu et al. (2020) compared the genomic prediction accuracy among high-throughput sequencing and SNP chips in broiler genotypes. Their study developed a new SNP marker screening method, the pre-marker selection (PMS), to test SNP markers' practicality for genomic prediction. Their results showed that the PMS method could improve genomic predictive breeding value accuracy. They stated that using high-throughput sequencing in native broiler populations to increase genomic markers can lead to more accurate predictions. The low cost of genotyping suggested that high-throughput sequencing may be a better alternative to SNP chips for genomic prediction in local broiler populations' selective breeding programs.

#### **4.6. Turkey (*Meleagris Gallopavo*)**

Despite its monetary value, studies on a turkey are few. Strillacci et al. (2019) examined 115 individuals from different breeds and geographies by using high-density SNP chips (Affymetrix 600K) to determine Copy Number Variants (CNVs). CNVs were clustered according to the individuals' breed populations and geographical origins in the study. Bernini et al. (2021) determined genetic diversity in seven autochthonous turkey breeds using the genome-wide characterisation of ROH (run of homozygosity) -rich regions. They stated that the breeds were genetically different; one (the Roma breed) was a mixture of the other breeds. Moreover, this study showed that more detailed information

about genetic variations in turkey breeds could be obtained using high-density SNP chips.

#### 4.7. Honey Bee (*Apis Mellifera*)

The honey bee is a eusocial model organism that can be examined regarding yield characteristics, especially honey, behavioural traits, and developmental differentiation. Spötter et al. (2016) used an Affymetrix 44K SNP to analyse SNPs associated with the honeycomb control to detect larvae parasitised by *Varroa*, the main parasite of the honey bee. In their study, thousands of honey bees were video-monitored; a hundred and twenty-two cases and the same number of control samples were collected. The detected SNP genotypes were examined to determine their dependence/independence between workers who exhibited hygienic behaviour and those who did not. After correcting for the false finding rate of p values, 6 SNP markers were very significant associations with the examined trait ( $\alpha < 0.01$ ). Detecting honeycomb control behaviour, observed as a measure of a honey bee's sensitivity to certain stimulants, and parasitised by *Varroa* larvae, is a key to breeding programs for *Varroa* resistance.

Jones et al. (2020) also reported 103,270 SNPs array for honey bees. The SNPs were selected based on their location within known candidate regions or genes and identified by sequencing whole-genomes of 61 drones from European countries. SNPs were primarily associated with hygienic behaviour-related traits and *Varroa destructor* resistance, honey production and docility. They suggested that this high-density SNP chip array is a robust and relatively simple way to study genomic selection, evolutionary genomics, and adaptation issues.

### 5. Conclusion

In this chapter, we summarised studies about GWAS and SNPs in cattle, water buffalo, sheep, goat, chicken, turkey, and honey bees to determine the relationships between SNPs and different properties, including yield, colour, meat quality, and resistance against stresses. SNP chips have been used for gene mapping and population genetic studies. However, gene transition on migration routes Colli et al. (2018) can be tracked, and populations can be clustered according to their breed and geography (Strillacci et al., 2019). While SNP chips are used in gene mapping and population genetic studies, they can also determine yield and behavioural traits. Therefore, SNP chips can make a significant contribution to breeding programs.

One of the main goals of animal breeding is to select the best parents for subsequent generations. There are many different and still not fully understood genetic mechanisms, such as gene expression regulations, the relationships between genes and their products, genome/environment interactions, etc. More sensitive, informative, and rapid scanning modules designed for epigenome maps and RNA sequences may soon replace livestock SNP chips. It is critical for the future of humanity to ensure that the livestock sector should aim to enhance the accuracy in the selection and coping with the malnutrition problems for the next generations.

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## CHAPTER III

# THE USE OF TRICHODERMA IN CONTROLLING APHIDS

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### 1. Introduction

Aphids (Hemiptera: Aphididae) are important agricultural pests that use many herbaceous and woody plants as hosts all over the world and damage their host directly or indirectly. The host plants of the phloem sap-sucking aphids are adversely affected during the stages of growth and development. It can cause delays in plant growth and product losses by inhibiting the photosynthesis process due to both yield reductions with the withdrawal of plant sap and the accumulation of honeydew. Indiscriminate use of insecticides in the fight against this pest causes various environmental problems such as pollution, as well as adverse effects on other beneficial insects and host plants. For this reason, it is emphasized that more reliable biological control methods or integrated control should be developed instead of chemical control in the control of these pests. Due to its positive effects on plant growth, *Trichoderma* species are considered to be a safer alternative method for controlling the aphids, directly or indirectly.

## 2. Aphids as Agricultural Pests

Insects are the major pests that cause losses in agricultural systems, crop productivity, and storage. Every agricultural product is attacked by different types of invasive insect/insects (Oliveira et al., 2014). Invasive insects are considered among the limiting factors, especially for the healthy growth of crop plants. Among insect pests, aphids are one of the most important pests of greenhouse and field crops, which have economic value throughout the world. Due to cyclical parthenogenetic reproduction and increasing their numbers in a short time, the aphid population increases rapidly, and thus, it can easily reach pest status (Hales et al., 1997). Aphids feeding on plant sap can cause major damage to crops by many mechanisms, either directly or indirectly (eg, being vectors of about 60% of plant viruses). As a result of the invasion of these insects, serious economic losses are experienced due to decreases in crop yields, and it is known that they cause an average of 35-40% product loss even in countries where effective control measures are implemented worldwide (Emden and Harrington, 2007; Asi et al., 2009). For example; In Pakistan and India, it has been shown that cabbage aphid can cause up to 70% product loss in cabbage when it is not properly controlled (Khan et al., 2015). Serious damage to the crop can occur through sap extraction, injection of various toxins, and/or virus contamination by aphids. Sometimes, during the feeding of aphids with plant sap, the crop yield decreases due to the secretion of honeydew, which prevents the photosynthesis process and causes a delay in plant growth, and sometimes the crop may be lost completely due to intense invasion (Embaby and Lotfy, 2016).

A wide variety of applications are used to control the aphids. Today, aphids are mostly tried to be controlled with chemical drug applications. For example; broad-spectrum insecticides containing pyrethroids, organophosphates, and neonicotinoids are used to reduce the damage caused by *Brevicoryne brassicae* (MAPA7). Accordingly, agricultural products in which insecticides are used (eg cabbage plant) are considered the agricultural products containing the most pesticides (Filgueira, 2008). Farmers mostly prefer chemical pesticide application in terms of application speed and ease. However, it has been stated that these chemical methods cause various environmental and health problems (Asi et al., 2009). Various environmental and agricultural sources of contamination are mentioned due to the indiscriminate use of insecticides, leading to the destruction of beneficial insects, the development of resistance in aphids, and the selection of naturally resistant aphids in subsequent generations

(Pacheco et al., 2017). It also causes problems such as environmental pollution and the accumulation of toxic residues in the natural ecosystem. For all these reasons, biological control is an alternative method to chemical control for insect pests (Akbari et al., 2014).

### 3. Fungi as Biocontrol Agents Against Insect Pests

Chemical pesticides which may affect the food products by leaving residual toxic compounds can also harm the plants out of target in the same environment as the crop plants. Many problems encountered with these pesticides make it necessary to develop alternatives to chemical applications. There are many microorganisms that interact with soil and plants, act as entomopathogens, are considered biopesticides against various agricultural pests, and constitute effective biocontrol agent candidates. Bacteria, viruses, protozoa, nematodes, and fungi have been reported as biopesticides that can be used in agriculture (Sindhu et al., 2017; Singh et al., 2019).

Fungi are filamentous eucaryotic microorganisms that have a wide range of use in biotechnological and industrial applications. They are considered as good sources of medicinal (ie. anticancer agents, antibiotics) and industrial (ie. enzymes) products, and are also used as biocontrol agents against various plant pests and pathogens. Moreover, various endophytic fungi are also used as bio-fertilizers or bioremediators (Hyde et al., 2019; Poveda, 2021). The use of entomopathogenic fungi as biopesticides is an alternative way that may substitute chemical-based pest control. The harmful effects of chemical pesticides can be reduced in agricultural fields by fungal biocontrol agents (Khosravi et al., 2015).

Some fungi species such as *Metarhizium*, *Beauveria*, *Hirsutella*, *Lecanicillium*, *Paecilomyces*, *Cordyceps*, *Ophiocordyceps*, *Torubiella*, *Isaria*, and *Pochonia* have the ability to infect and kill arthropods, which are most frequently utilized in agriculture, recognized as entomopathogens. Since the lytic enzymes (proteases, lipases, and chitinases) and adhesins are required, entomopathogenic fungi infect their hosts by penetrating directly into the cuticle. Next, the fungus begins to form and spread new conidia, defeating the insect's immune system. During the process, a wide range and variety of insecticidal secondary metabolites are synthesized by entomopathogenic fungi throughout the completion of their life cycle (Qu and Wang, 2018; Litwin et al., 2020).

Analyzing the possible damage that occurs on the natural enemies is important while applying mycopesticides against agricultural pests as the development of Integrated Pest Management (IPM) depends on the use

of different biological sources involving predators, microorganisms, and/or parasitoids. In this aspect, some studies have indicated that the activity of predatory insects and parasitoids can be significantly reduced by the various entomopathogenic fungi (Vazquez, 2019; Abbas, 2020).

The epiphytic effects of entomopathogenic fungi have been extensively studied in the biocontrol of pests and plant disease prevention studies (Lozano-Tovar et al., 2017). However, studies conducted in recent years on endophytic fungi as entomopathogens have also increased. The fungal species which colonize in plant tissues during most or all of their life cycle causing any significant damage to the host plant are defined as endophytic fungi (Russell et al., 2011). They relate to many of the plants that naturally exist in the ecosystem and are considered to have important implications for plant growth. By providing many beneficial effects to the host plants, such as promoting plant growth, inhibiting pathogenic organisms, removal of soil pollutants, and increasing the tolerance of the host plant against extreme temperature, water, and salt stress these microorganisms have attracted researchers in past few years (Quesada-Moraga et al., 2009; Kauppinen et al., 2016).

Some studies have indicated that endophytic fungi are able to protect host plants against pathogens and herbivores (Vega et al., 2008). It may also provide long-term insect control without harming the environment or non-target organisms. These fungi can be easily isolated from the soil under natural conditions and are broad-spectrum, causing deadly infections (de Faria and Wraight, 2007). The use of fungi has numerous advantages over conventional pesticides when the safety of food, humans, and other non-target organisms is taken into account. Unlike other microbial pathogens such as bacteria, viruses, and protozoa that infect the host by ingestion; It invades the host by penetrating the outer integument and shows active parasitism ability, using the insect's body as a food source for the formation of new conidia (Poveda, 2021). Following the death of the host, the fungus can still continue to digest the cadaver and grow to produce spores for infecting other host insects (Khaleil et al., 2016). The efficiency of entomopathogenic fungi is mostly related to the species and the virulence of the strain (Shah et al., 2005). Various entomopathogenic fungal species, such as *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Lecanicillium lecanii* (Zare and Gams), *Beauveria bassiana* (Bals.-Criv.) Vuill., *Purpureocillium lilacinum* (Thom), *Paecilomyces farinosus*, *Paecilomyces lilacinus*, and *Paecilomyces fumosoroseus* have demonstrated pathogenic effects against tested several insect pests, including aphids (Islam et al., 2021).

#### 4. Use of *Trichoderma* as Biocontrol Agents

About a hundred described species of the genus *Trichoderma*, which is in the group of endophytic fungi, are widely spread through the world with the ability to rapidly grow using various substrates while tolerating the different environmental conditions and pollutants that exist in the soil (Mishra et al., 2016; Hu et al., 2020). Having so many beneficial effects during the growth and development of the host plants *Trichoderma* species have attracted great interest in recent years (Woo et al., 2014; Alfiky and Weisskopf, 2021). As one of the most frequently isolated soil fungi, the *Trichoderma* genus constitutes one of the most preferred biocontrol agents for studies. It is generally found in plant rhizosphere systems. The high adaptation ability to different environmental conditions and nutritional compatibility allows the members of the *Trichoderma* genus to exist in a wide variety of ecological niches (Harman, 2006).

While *Trichoderma* species take place as plant growth promoters and biocontrol agents in agriculture, they have become a powerful tool with economic benefits by using them for various purposes (ie. as enzyme producers) in different industrial processes (Jangir et al., 2017). The use of *Trichoderma* in biotechnology has gradually increased in the past few years due to their support in plant growth and tolerance increment against abiotic stress factors in agriculture (Poveda et al., 2019a; Poveda et al., 2020), their importance as biofertilizers (Zhang et al., 2018), mycoremediators (Solanki et al., 2019), and as gene resources (Poveda et al., 2019b).

*Trichoderma* species that belong to the Hypocreales order of Ascomycota can be easily isolated from several sources that include plant roots, leaves and stems, soil, and decaying organic plant material (Howell, 2003). Different nutrient media such as malt agar (MA), Glucose Peptone Yeast Broth (GPYB), potato dextrose agar (PDA), and Czapek dox agar (CDA) are suitable for *Trichoderma* species to grow rapidly and produce different shades of conidia/spores characterized by green color (Chaverri et al., 2003). *Trichoderma* isolates are a good source of antibiotics with different effects against various pathogenic microorganisms. For example, azaphilone and butenolide isolated from *Trichoderma harzianum* have been reported to have potent antifungal activity against different yeasts. In addition to its use as a potent bio-pesticide, it has also been suggested to be used as a biocontrol agent against soil-borne fungi and other bacterial pathogens (Rahim and Iqbal, 2019). Generally, *Trichoderma* is considered to be an economical and environmentally friendly plant growth

promoter (Harman et al., 2019; Patel et al., 2019). Along with suppressing plant diseases, it encourages plant growth by increasing water and mineral intake. Following the colonization of *Trichoderma*, there are significant changes in the plant proteome and metabolome, thus increasing the plant's productivity and tolerance to abiotic and biotic stresses (Shoresh et al., 2019).

*Trichoderma* species can be inoculated to crops in a variety of ways (eg, application to pre-sowing seed or propagation material, application as root soaking or immersion, incorporation into the soil during seeding or transplant). Woo et al. (2014) determined that the most successful fungal colonization was the direct application to seeds and roots. *T. reesei*, *T. atroviride*, and *T. virens* are the most widely used *Trichoderma* species in agriculture as biocontrol agents (Kubicek et al., 2011).

Most of the studies with *Trichoderma* species are conducted to investigate in vitro insecticidal effects of the fungal spores. *T. harzianum* and *T. longibrachiatum* parasitized the adults of *Bemisia tabaci* and *Cimex hemipterus*, causing mortality rates of 40% in 5 days and 90% in 14 days. Likewise, after 15 days of application, different *Trichoderma* species, the bean weevil (*Acanthoscelides obtectus*), the coconut palm rhinoceros beetle (*Oryctes rhinoceros*) and the vineyard borer (*Xylotrechus arvicola*) adults, as well as their larvae and eggs, have been reported to have nearly 100% mortality. It is reported that the lesser grain borer (*Rhyzopertha dominica*) adults showed a mortality rate of 94% in the presence of *T. album* applied wheat grains in 7 days. In addition, the application of spores of *T. longibrachiatum* to the leaves in the field causes the death of approximately 50% of the brinjal borer *Leucinodes orbonalis*, while it has the ability to increase the yield of eggplant by 56% (Poveda, 2021).

## 5. Use of *Trichoderma* Against Various Aphid Species

Many entomopathogenic fungi are effectively used in the fight against various insect groups. In recent years, some species belonging to the genus *Trichoderma* have been reported to be effective in controlling aphids (Islam et al., 2022). Aphids are infected by a large number of entomopathogenic fungi. Therefore, studies on finding novel microbial agents to meet the needs for biological control of pests in agricultural fields are important. For biological control of the local pests isolation and screening of more local fungal strains with higher insecticidal activity and adaptation have great value (Fournier and Brodeur 2000; Sewify et al., 2000).

*Trichoderma* species can synthesize secondary metabolites with great insecticidal potential in their environment. In this sense, the most studied species is *T. harzianum*, which produces various metabolites. In different insect groups, including aphids (for example, wheat aphids *Diuraphis noxia* and *Schizaphis graminum*, and cotton aphid *Aphis gossypii*) it can cause 100% mortality in about 15 days (Ganassi et al., 2000; Rahim and Iqbal, 2019).

It has been reported that *T. harzianum* is pathogenic against *Aphis gossypii* and can be used to control this pest (Nawaz et al., 2020; Mukherjee et al., 2020). *Trichoderma* species have been shown in studies to be effective in the control of wheat aphid *Schizaphis graminum* Rondani (Hemiptera: Aphididae) and cabbage aphid, *Brevicoryne brassicae* (Linnaeus, 1758) (Hemiptera: Aphididae) (Pochecho et al., 2020; Khaskheli et al., 2019). It has been reported that the penetration of *Macrosiphum euphorbiae* (Thomas, 1878), which prefers tomato plants as hosts, can be significantly reduced by using the P1 strain of *T. atroviride* (Coppola et al., 2019a).

*Trichoderma* may also act indirectly as a plant endophyte or a mycoparasite through activation of systemic plant defense responses, attraction to natural enemies, or parasitism of insect symbiotic microorganisms. Through the colonization of the roots, *Trichoderma* can trigger plant defense systems systemically by the stimulation of plant hormones salicylic acid (SA) and jasmonic acid (JA) against the attack of pathogens and pests. It has been reported that *T. atroviride*, *T. longibrachiatum*, and *T. harzianum* colonize the roots of tomato plants and create SA-mediated resistance, causing a mortality rate of 100% of the potato aphid *Macrosiphum euphorbiae* individuals within 25 days (Coppola et al., 2019a; Coppola et al., 2019b).

In addition to the plant's response to pathogens, *Trichoderma* can also induce plant defense against insect attack by activation of plant defense-related pathways (Rasmann et al., 2017). Plant metabolic changes caused by *T. harzianum* were evaluated in a model system consisting of the tomato plant, aphid *Macrosiphum euphorbiae*, and parasitoid *Aphidius ervi*. It has been reported that *T. harzianum* T22 application mediates the production of some volatile organic compounds (VOCs), which are known to attract parasitoid flight (Coppola et al., 2017). VOCs emitted by other microorganisms have been shown to affect the secretion of antibacterial and antifungal molecules in *Trichoderma* (Li et al., 2019). The co-occurrence of *T. harzianum* in plant roots and aphids in leaves caused parasitoid attraction to infested plants, and plants were shown to produce a different VOC profile in the presence of both *Trichoderma* and aphids than

when both were alone. A similar finding was obtained with *T. longibrachiatum*, which modifies VOCs released by tomato plants, increasing attractiveness to natural enemies of the aphid *M. euphorbiae* (Battaglia et al., 2013).

*Trichoderma* species parasitize insects at all stages, including non-feeding, pupa, egg stages, winged and wingless stages. The effects of some strains of the genus *Trichoderma* against *Schizaphis graminum*, one of the most important pests of cereal crops in the world, are examined. Ganassi et al. (2000) observed different effects of two different strains of *T. harzianum* on aphids in their study. The ITEM 908 strain has been reported to cause a significant reduction in the survival rate of the larvae within the first 10 minutes of the experiment. The survival rate of the nymphs was significantly reduced within the first hour, while the survival of the wingless was significantly reduced compared to the controls at the eighteenth hour of the experiment. The application with the ITEM 910 strain caused a significant decrease in the survival of the larvae in the first 3 hours, while it had no lethal effect on the wingless individuals, and a decrease in the survival of the nymphs was similar to the control group. Thus, it has been demonstrated that with *Trichoderma* applications, all three aphid groups of *S. graminum* (15-hour larvae, 5-days old nymphs, and wingless forms) can be controlled significantly.

A positive correlation was observed between time and concentration in the applications of *Trichoderma* strains. Nawaz et al. (2020) investigated the effect of different concentrations and application times of *Trichoderma* and *Bacillus thuringiensis* on cotton aphid *Aphis gossypii* (Glover), and it was determined that *Trichoderma* sp. had a 73% mortality rate. With this result, it has been demonstrated that these microbial organisms can be included in IPM programs for effective control of insect pests.

Mukherjee and Ghosh (2023) compared the efficacy of *T. harzianum*, *B. Bassiana*, and malathion (insecticide) applications in the control of *Aphis gossypii*. After 48 hours of application, the LD50 dose of *T. harzianum* was determined as  $1.2 \times 10^5$  mL<sup>-1</sup> spore, while the LD50 dose of *B. bassiana* was determined as  $1.0 \times 10^6$  mL<sup>-1</sup> spore. Considering the LT50 values, it was determined that *T. harzianum* (47.70 hours) exhibited a lower effective time than *B. bassiana* (57.53 hours). It was revealed that the application of *T. harzianum* in agricultural areas showed a decrease of approximately 32% in the aphid population, while the application of malathion showed a decrease of approximately 24%. The results showed that *T. harzianum* was more effective than *B. bassiana* in controlling this aphid; It suggests that *T. harzianum* should

replace the hazardous chemical pesticide malathion for environmentally friendly biological control of aphids.

## 6. Conclusion and Recommendations

Insect pests cause serious losses in crop productivity worldwide. In order to minimize these losses, pesticide applications are performed. However, chemicals and fertilizers used in agriculture threaten human and environmental health. For this reason, the search for more sustainable and environmentally friendly solutions has led to the idea that various organisms can be used as biocontrol agents. Insecticides used to control aphids both harm non-target organisms and host plants, and cause environmental problems such as pollution. Therefore, it is essential to develop more reliable biological control methods instead of chemical control in controlling these pests.

It is clear that *Trichoderma* species, which are effective in the production of various metabolites by activating the plant defense system, are a safer alternative way, directly or indirectly, for the control of aphids. The *Trichoderma* genus, which has different biological properties, controls some pathogenic fungi, insects, and nematodes, and also stimulates plant growth by activating the plant's defense mechanism, making this fungus a good option as a biocontrol agent.

Studies conducted in recent years also report that the effectiveness of *Trichoderma* on insect pests has mortality rates of up to 100% both in the laboratory and in the field. However, most of these studies are performed in a controlled environment such as a laboratory or greenhouse. In this context, laboratory trials may not accurately reflect soil microbiota and field conditions. To confirm the results obtained, the trials should be done mostly in field conditions. For exploring the possible effects of *Trichoderma* treatments on other natural enemies, non-harmful insects, and native entomopathogens parallel studies should also be designed.

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## CHAPTER IV

# AGRICULTURE IN THE AGE OF GLOBALIZATION: POSSIBILITIES AND CHALLENGES, AND THE ROLE OF BIOTECHNOLOGY

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### **1. Introduction**

Agriculture is an essential economic activity that has supplied humanity with food, fiber, and other vital resources throughout history. Yet, globalization has brought about considerable changes in the agriculture industry. Among the sector's possibilities and problems include shifting production and consumption patterns, market access, trade regulations, and environmental concerns. This chapter examines the effects of globalization on agriculture, as well as the opportunities and obstacles the sector faces in a globalized world.

Globalization has had a considerable impact on the agricultural sector, which has historically been a major contribution to the economies of many nations, especially those in development. Nonetheless, the expansion of globalization has offered new challenges and opportunities. Agriculture greatly contributes to global food security, livelihoods, and economic growth (IFAD 2021; USDA 2021). The globalization of commerce, investment, and technology has altered the agriculture industry, generating new opportunities and problems for farmers, agribusinesses, and government officials (Lee 2017; Lusigi 2016; Shrestha & Upadhyay 2015). This article explores the effects of globalization on agriculture, the challenges faced by farmers, and the emerging prospects.

It focuses on sustainable agriculture, rural development, and food security and provides an overview of agricultural globalization's trends, drivers, and repercussions. The agricultural, forestry, and aquaculture sciences are essential to the maintenance of the global food and natural resource systems. As a result of globalization's expanding interconnectedness, a number of regions confront new challenges and opportunities. In addition to a rise in demand for food, wood products, and seafood, globalization has generated new markets for these goods. Yet, it has exacerbated competition, environmental pressures, and socioeconomic inequality. According to the United Nations' Food and Agriculture Organization (FAO), the global population will reach 9.7 billion by 2050, needing a 70 percent increase in food production (FAO 2017; UN DESA 2017). Significant environmental issues, such as climate change, land degradation, and biodiversity loss, threaten the sustainability of food and natural resource systems. To address these difficulties, scientists, policymakers, and stakeholders must engage in cross-sectoral cooperation and adopt innovative techniques. In a worldwide world, agriculture, forestry, and aquaculture sciences play a crucial role in addressing these difficulties and guaranteeing sustainable development. They can aid in the improvement of food security, conservation of natural resources, mitigation of climate change, promotion of rural development, and reduction of poverty. This requires a strategy that integrates scientific knowledge with social, economic, and environmental factors (UNDP 2020; IPBES 2019; FAO 2021; The Royal Society 2009).

Agriculture faces new difficulties and opportunities as a result of globalization, which has created a complex environment for farmers, governments, and other stakeholders to navigate. This section discusses some of the greatest difficulties and opportunities facing agriculture in a globalized society. They include greater competition in agricultural markets, which can put farmers under pressure to lower their prices or expand their output, leading in decreased incomes for small-scale farmers.

## **2. Climate Change**

The consequences of climate change on world food output are anticipated to be both good and detrimental (Hartmann et al., 2013) Climate change is expected to impact agricultural output by variations in temperature, precipitation, and extreme weather, which in turn impact crop yields, soil fertility, and livestock production (IPCC 2014), Intergovernmental Panel on Climate Change Climate change is impacting agriculture in numerous ways, including changes in rainfall

patterns, rising temperatures, and an increase in the frequency of extreme weather events, which can diminish crop yields and livestock production (UNEP 2021).

### **3. Land Degradation**

To reduce the effects of climate change, this necessitates that farmers modify their operations and adopt new technologies. Land degradation, which includes soil erosion and depletion, is a growing problem for agriculture, especially in developing nations, and can reduce the productivity of agricultural land, making it more difficult for farmers to meet the rising demand for food. Globalization has opened new markets for agricultural products, enabling farmers to extend their client base and income. This has benefited the livelihoods of small-scale farmers and helped to rural economic development (Pretty et al., 2011). Precision agriculture is a big advantage of technical improvements. Farmers may maximize inputs and save waste by collecting data on soil conditions, crop growth, and weather using sensors, GPS, and drones. This has improved agricultural productivity and sustainability (Lebel et al., 2013).

### **4. Sustainable Agriculture**

Globalization has increased interest in sustainable agriculture, supporting the development of practices such as agroforestry and conservation agriculture that lessen agriculture's environmental impact (Godfray et al. 2010). As the global population continues to rise, there is an increasing need to produce more food while limiting agriculture's environmental impact. Innovations in technology play a crucial role in promoting sustainable agriculture (Kumar 2019; Pimentel and Giampietro 2014). In additionally this section, we shall examine, with supporting references, some of the latest technologies and technological developments that are boosting sustainable agriculture.

#### ***4.1. Precision Agriculture***

Precision agriculture is an example of a technology that has transformed agriculture. Farmers may optimize inputs, boost agricultural yields, decrease waste, and reduce environmental effect by collecting data on soil conditions, crop growth, and weather (FAO, 2021).

#### ***4.2. Agroforestry and Conservation Agriculture***

Agroforestry, which involves introducing trees into agricultural landscapes to increase soil health, minimize soil erosion, and reduce greenhouse gas

emissions, is another sustainable approach (Leakey et al., 2012). Conservation agriculture is a land-use strategy characterized by low soil disturbance, the maintenance of soil cover, and crop rotation, which results in better soil health, decreased soil erosion, and increased biodiversity (Kassam et al. 2019; Pittelkow et al. 2015; Verhulst et al. 2010).

### **5. Aquaponics and Opportunities for Farmers**

Aquaponics is yet another unique technology that combines aquaculture with hydroponics in a closed-loop system to reduce water use and fertilizer contamination while simultaneously growing crops (Rakocy et al., 2006). Agriculture has been affected both positively and negatively by globalization. The expansion of agricultural product trading as a result of trade liberalization policies has created new opportunities for farmers in some nations while posing new obstacles in others (Anderson 2009; Carter & Mohapatra 2013; Mishra & Kumar 2018; Schmitz & Schmitz 2015). Farmers in impoverished nations have been forced to compete with larger, more efficient producers from developed nations, resulting in price decreases and market share declines for small-scale farmers (IFAD 2016; Shiva 2016). The rise of global supply chains has increased the pressure on farmers to produce at cheaper costs, which has raised issues over labor exploitation and environmental damage (Fuchs et al. 2011; Gereffi et al 2005; Goldsmith et al. 2014; Jaffee et al. 2001). Notwithstanding the difficulties posed by globalization, farmers have discovered new opportunities. The expansion of global supply chains has provided new markets for small-scale producers to explore, and new technology have enabled farmers to increase productivity and efficiency, so enhancing their ability to compete on the global market. Trade liberalization, technological innovation, urbanization, and population changes all contribute to agricultural globalization.

### **6. Challenges for Farmers**

They have generated new risks and uncertainties for farmers and consumers, as well as new potential and challenges for small-scale farmers, women, and youth. The introduction of new technologies has increased production, efficiency, and profitability, but has also prompted worries regarding their environmental and social impacts. The globalization of agriculture offers several prospects for sustainable agriculture. Among these prospects are climate-smart and resource-efficient methods, the promotion of value-added and high-value

crops, and the integration of smallholder farmers into global value chains (FAO 2019; UNCTAD 2013; IFAD 2016). Climate-smart agricultural approaches, such as conservation agriculture, agro forestry, and precision agriculture, can enhance soil health, conserve water, and reduce greenhouse gas emissions (Lal 2019; Pretty et al. 2018). Smallholder farmers can increase their income and competitiveness by promoting value-added and high-value crops, such as organic, fair-trade, and specialty products, while minimizing their dependency on commodity crops. Through contract farming, farmer associations, and e-commerce platforms, including smallholder farmers into global value chains can enable access to markets, cash, and information. Yet, agricultural globalization poses a number of challenges to rural development and food security (Rao & Mehta 2017; German et al. 2017; Rajeev & Menon 2016; Wheeler & Black 2003). The concentration of market power and hazards in the hands of a few numbers of agribusinesses, traders, and retailers can diminish the bargaining power and income of smallholder farmers and increase market volatility and uncertainty. The displacement of smallholder farmers due to land grabbing, urbanization, and climate change can result in social and economic isolation and food insecurity (Gabriel et al. 2015; Amanor 2015; Warner & Köhler 2012; Mugera & Hertel 2011). Degradation of natural resources such as soil, water, and biodiversity threaten the resilience and sustainability of agriculture and the food security of future generations. Agriculture has been substantially impacted by globalization (World Bank (2016)). Farmers now have access to new markets and technologies, which has increased their productivity and profitability. It has also brought challenges, such as greater competition, evolving client preferences, and environmental degradation. Access to markets is usually constrained by trade barriers such as tariffs, quotas, and non-tariff barriers, making international competition tough for farmers (WTO 2019). Nonetheless, globalization has facilitated the dissemination of new agricultural technologies and best practices, such as precision agriculture, biotechnology, and sustainable farming methods. Technology has helped farmers increase productivity and efficiency, cut expenses, and improve product quality. In addition to soil deterioration, water scarcity, and climate change, globalization has brought forth environmental hurdles for agriculture (WWF 2020). To ensure the long-term survival of agriculture, innovative solutions and sustainable farming practices are required to meet these challenges. Agriculture faces numerous challenges and opportunities in the era of globalization. The agricultural sector is substantially influenced by trade policies and agreements. For instance, subsidies, tariffs, and

non-tariff obstacles can distort global agricultural markets, leading to increased competition and decreased profitability for farmers. However, trade agreements can also increase farmers' access to new markets and technologies.

### **7. Globalization and Agriculture**

Globalization has impacted consumer expectations for agricultural products such as locally grown and organic foods. This mobility presents both challenges and opportunities for farmers, who must adapt to altering market demands while competing with larger, more established producers. Due to environmental concerns, sustainable agricultural practices such as conservation tillage, cover crops, crop rotation, and reduced pesticide use are gaining importance. These techniques can improve soil health, increase crop productivity, and lessen environmental impacts.

### **8. Biotechnology in Sustainable Agriculture**

Due to its capacity to increase crop productivity and lessen the environmental impact of farming techniques, biotechnology is a crucial tool for sustainable agriculture. This chapter will provide references to discuss the function and significance of biotechnology in sustainable agriculture in a globalized world.

Biotechnology has enabled the development of genetically modified (GM) crops, which offer numerous advantages such as resistance to pests and diseases, drought tolerance, and increased yield. According to (Brookes & Barfoot, 2020): Numerous nations have adopted GM crops, which have increased food security and decreased pesticide use. By increasing microbial activity and nutrient cycling, biotechnology can also benefit soil health. Biochar, microbial inoculants, and biofertilizers can improve soil health and fertility (Verma et al., 2019). In addition, biotechnology can aid to climate change mitigation by lowering greenhouse gas emissions. Biotechnology can be used to generate crops that use nitrogen more efficiently, hence reducing the demand for nitrogen fertilizers, which are a substantial source of nitrous oxide emissions (Kramer et al., 2020). Moreover, biotechnology can be utilized to improve the sustainability of aquaculture by using genetic selection to generate disease-resistant and faster-growing fish, hence lowering the need for antibiotics and other chemicals (Nhu et al., 2019).

## 9. Conclusion

Agriculture, rural development, and food security face both opportunities and problems in a globalized world. Policies and tactics that promote inclusive, equitable, and sustainable development are crucial for maximizing opportunities and removing barriers. These policies and efforts should prioritize the empowerment of smallholder farmers, the promotion of innovation and technology transfer, the improvement of infrastructure and logistics, and the enhancement of governance and accountability. In a worldwide world, the future of agriculture is influenced by population increase, climate change, technology advancements, and changes in consumer tastes. Farmers must continue to adapt to fluctuating market demands, technological developments, and environmental concerns. In a globalized environment, sustainable farming techniques, improved market access, and innovation in response to altering consumer demands can help farmers flourish. Population growth will continue to place pressure on global food systems, necessitating that farmers produce more food with less resources and in a sustainable manner. Precision agriculture, which employs data and technology to enhance output, reduce waste, and boost yields, will receive more attention. Due to climate change, changes in temperature, precipitation patterns, and extreme weather events will have a considerable impact on crop yields and production. To adapt to these changes, farmers will need drought-resistant crops, improved irrigation techniques, and other climate-smart agricultural practices. Drones, robotics, and artificial intelligence (AI) will play a major role in agriculture, allowing farmers to automate operations, reduce labor costs, and increase production. With the use of these technologies, farmers will be able to keep a closer eye on their crops, identify problems sooner, and make better decisions regarding fertilization, irrigation, and pest control. The future of agriculture will be influenced by consumer demand for organic, locally sourced, and sustainably produced food. The food supply chain will become more transparent and easier to track, and the market for heritage crops and other specialty items will expand. In a globalizing society, biotechnology plays a crucial role in promoting sustainable agriculture. Biotechnologies such as genetic engineering, microbial biotechnology, precision breeding, and biopesticides can increase agricultural yields, improve nutrient uptake, and reduce environmental impact. The biotechnology of plants has the potential to play a significant role in the globalization of agriculture. Creating crops that are more resistant to climatic difficulties such as drought, heat, and salt can help mitigate climate

change. Plant biotechnology can promote food security, improve sustainability, and address concerns of social and ethical responsibility.

In conclusion, it is vital for the future of agriculture in a globalized world to strike a balance between supplying the expanding need for food, preserving the environment, and protecting the welfare of farmers and rural communities. The adoption of sustainable agricultural methods, innovation, and the application of technologies such as biotechnology can aid in achieving this equilibrium.

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## CHAPTER V

# AN EXAMPLE TO SPI DROUGHT ANALYSIS OF THE DROUGHT FUTURE: VAN, TURKEY

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### 1. Introduction

**D**rought is a natural hazard that occurs when water is deficient, either in the form of precipitation or soil moisture, to meet the needs of plants, animals, and humans. Drought can have significant impacts on various sectors, such as agriculture, forestry, water resources, and energy, as well as on the environment and human health (Wilhite, 2000). Drought can be classified based on its duration, severity, and extent. It can be long-term, lasting for several months or years, or short-term, lasting for a few weeks or days. Drought severity is typically measured using drought indices, such as the Standardized Precipitation Index (SPI) or the Palmer Drought Severity Index (PDSI), and so on (Quiring, 2009). Drought extent refers to the spatial coverage of drought, which can range from local to regional to global (Wilhite, 1985). This can be caused by a variety of factors, including natural climate variabilities, such as El Niño, human activities, and land use change with the over-extraction of groundwater (Awange et al., 2014). Drought can be predicted and monitored using a variety of tools and techniques, such as weather forecasts, satellite data, and ground-based observations (Roushangar, et al., 2021). Effective drought management and mitigation strategies can help to reduce the impacts of drought

on society and the environment. These strategies may include water conservation measures, the use of drought-tolerant crops and irrigation techniques, and the development of drought early warning systems (Zhang and Kite, 1999).

The Standardized Precipitation Index (SPI) is a meteorological drought index that is used to quantify the severity and duration of drought conditions. It is based on the probability of dryness, which is calculated using the distribution of precipitation over a given period of time (McKee et al., 1993). The SPI is commonly used by hydrologists, meteorologists, and water resource managers to monitor and forecast drought conditions, and to assess the potential impacts of drought on various sectors, such as agriculture, forestry, and water resources (Naresh et al., 2009). The probability of dryness is transformed into an index value using a logarithmic scale, which allows for the index to be expressed as a standardized score ranging from  $\leq -2$  (severe drought) to  $+2 \geq$  (extremely humid). A value of 0 indicates normal precipitation conditions. The SPI can be calculated for different time scales, such as monthly, quarterly, or annual, and for different locations, such as a single weather station or a region, and has been widely used and validated in a variety of regions and climates, which is a useful tool for identifying and characterizing drought conditions (McKee et al., 1993). It has been applied in numerous studies and projects, including drought monitoring and early warning systems, water resources management, and crop yield prediction (Zhang and Kite, 1999).

The DrinC software uses satellite-based precipitation estimates and other climate data, such as temperature and humidity, to calculate the SPI for various time scales (e.g., monthly, quarterly, annual) and locations. It also provides various visualizations and maps of the calculated SPI values, which can be used to assess the spatial and temporal evolution of drought conditions.

In Turkey, drought has been a recurrent problem, particularly in the last few decades. According to a study published in the journal *Environmental Earth Sciences*, drought conditions in Turkey have become more frequent and severe in recent years, as indicated by various drought indices like SPI (Yücel and Aksoy, 2017). In Van province, Turkey, drought has been a recurrent problem, as drought conditions in Van province have become more frequent and severe in recent years, due to a combination of factors, including increasing temperatures, decreasing precipitation, and increasing evapotranspiration. These factors have resulted in reduced soil moisture and water availability, which have had negative impacts on agriculture and forestry, as well as on the environment and human health (Öztürk and Öztürk 2017).

In this study, Van province meteorological precipitation data between 1980 to 2020 years that comprise 1-, 3-, 6- and 12 months was analyzed by using Standard Precipitation Index through DrinC software.

## 2. Material and Method

### 2.1. Material

#### 2.1.1. Research Data and Province

In this study, the drought analysis of Van province was done by using DrinC software through SPI implementation. Data was taken from the Turkish State Meteorological Service 17172 station number, between 1980-2020 years range.

**Table 2.1.** Van province geographical information and date range

Station Name	Altitude	Date Range	Latitude	Longitude
Van	1812	1980-2020	38.499817	43.378143.



**Figure 2.1.** The geographical location of Turkey and Van province

According to the Turkish State Meteorological Service, the average annual precipitation for Van province is about 392.8 millimeters (mm). This amount is calculated by taking the mean of precipitation measurements from various weather stations in the area over several years.

## 2.2. Method

### 2.2.1. Standardized Precipitation Index

In the study, the Standardized Precipitation Index (SPI) method, which converts the precipitation parameter into a single numerical value, was used to define the drought of regions with different climates. This method was first

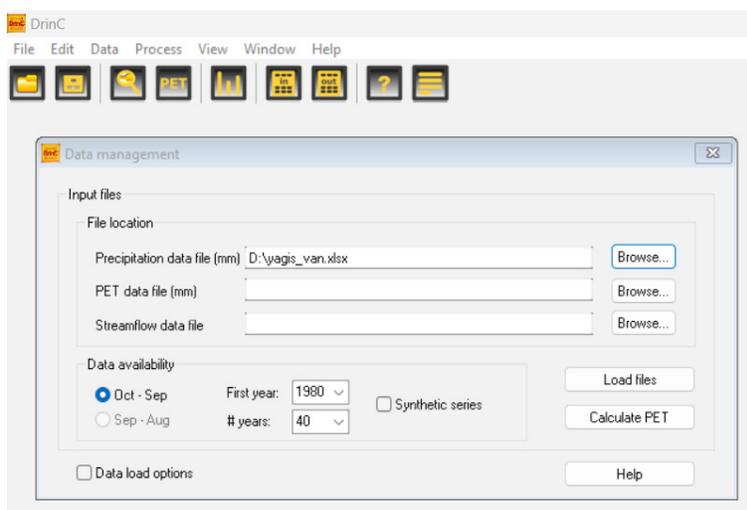
described by McKee et al. (1993) and obtained by dividing the difference of precipitation from the mean by the standard deviation in a selected time period with the following equation.

**Table 2.2.** Classification of SPI index values

SPI Interval	Drought Severity Level
$\leq -2.0$	Severe drought
-1.5 to -2.0	Extreme drought
-1.0 to -1.5	Moderate drought
-0.5 to -1.0	Mild drought
-0.49 to 0.49	Normal
0.5 to 1.0	No drought
1.0 to 1.5	Above normal
1.5 to 2.0	Much above normal

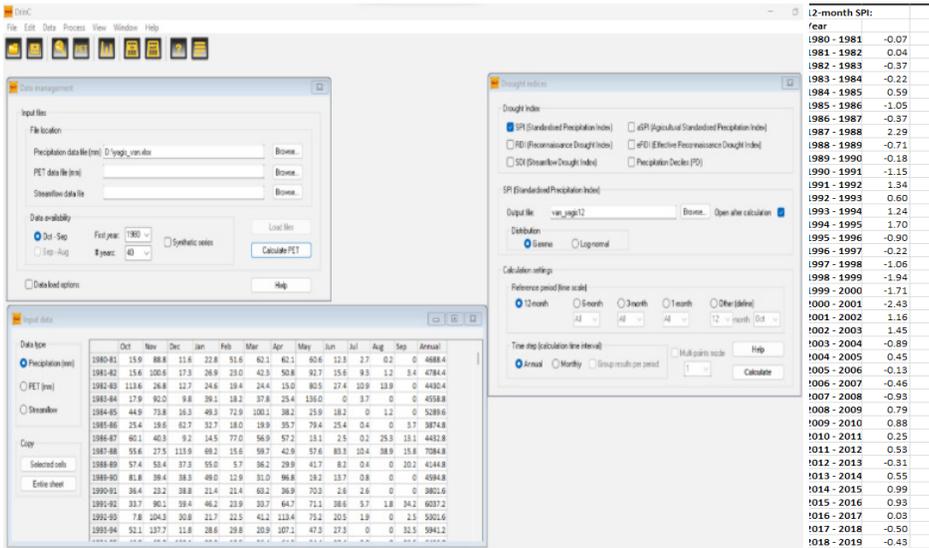
### 2.2.2. DrinC Software Usage

The file containing the precipitation data is uploaded to the “Data management” section in the “Data” section from the top menu. The data in the file is sorted by “Oct-Sep”, that is, from October to September. The year that the acquired data starts with and how many years it covers are specified in the “first year” and “#years” sections.



**Figure 2.2.** Data upload on DrinC

The preview of this data can be viewed in the “data view” section. After confirming that the data is correct, clicking the “calculate” button opens the window titled “drought incidents”. The index and settings required to be calculated in the pop-up window are made. In the “Output file” section, the name of the file from which the data will be calculated must be written. Data can be modified by month before the computation is completed and months not specified in the button can be calculated by writing in the “others” section.



**Figure 2.3.** Index selection, date range, and calculation

For drought analysis, monthly precipitation data measured between 1980 and 2020 at Van station 17172 located in the Middle Euphrates Section of the Eastern Anatolia Region of Turkey were used. SPI values were calculated according to the gamma distribution of the precipitation data using the DrinC program.

Years	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
1980 - 1981	-0.91	1.17	-1.14	-0.59	0.86	0.79	0.46	0.56	-0.02	-0.02	-0.10	-0.67
1981 - 1982	-0.93	1.40	-0.78	-0.30	-0.31	-0.21	0.01	1.43	0.20	0.77	0.12	-0.23
1982 - 1983	1.55	-0.61	-1.06	-0.46	-0.52	-1.45	-2.17	1.13	0.82	0.91	1.23	-0.67
1983 - 1984	-0.80	1.23	-1.27	0.44	-0.60	-0.48	-1.33	2.34	-1.65	0.14	-0.19	-0.67
1984 - 1985	0.19	0.84	-0.83	0.94	1.46	2.24	-0.57	-0.85	0.36	-0.93	0.12	-0.67
1985 - 1986	-0.45	-0.95	0.86	0.07	-0.61	-1.85	-0.70	1.10	0.73	-0.63	-0.19	-0.20
1986 - 1987	0.57	-0.09	-1.31	-1.33	1.57	0.55	0.27	-1.74	-1.08	-0.74	1.81	0.39
1987 - 1988	0.47	-0.58	1.93	1.75	-0.77	0.68	-0.34	0.46	2.52	0.87	2.34	0.52
1988 - 1989	0.51	0.32	0.11	1.19	-1.77	-0.58	-1.04	-0.11	-0.36	-0.63	-0.19	0.70
1989 - 1990	1.02	-0.12	0.14	0.92	-0.98	-0.93	1.56	-1.26	0.08	-0.47	-0.19	-0.67
1990 - 1991	-0.06	-0.77	0.16	-0.70	-0.40	0.84	-0.64	0.85	-1.06	-0.04	-0.19	-0.67
1991 - 1992	-0.15	1.20	0.77	0.79	-0.26	-0.75	0.55	0.87	1.27	0.41	0.21	1.16
1992 - 1993	-1.50	1.47	-0.14	-0.68	-0.34	-0.27	2.00	0.98	0.49	-0.18	-0.19	-0.31
1993 - 1994	0.38	2.05	-1.12	-0.18	0.03	-1.76	1.83	0.11	0.82	-0.93	-0.19	1.11
1994 - 1995	0.22	0.63	2.33	-0.74	-1.20	-0.57	0.54	-0.42	0.48	0.03	-0.19	2.47
1995 - 1996	0.13	-0.77	-0.98	-1.11	0.37	1.54	-0.09	-1.12	-0.71	0.53	-0.02	1.22
1996 - 1997	0.63	-1.63	0.92	-0.53	-0.33	1.31	-0.88	-1.01	0.71	2.09	-0.19	0.27
1997 - 1998	0.49	-1.47	1.03	-0.10	0.41	-1.27	-0.40	-0.35	-0.14	-0.41	0.12	-0.67
1998 - 1999	-3.38	-1.21	0.72	-2.16	-0.21	-0.01	-0.06	-0.10	-0.44	-0.93	0.27	0.58
1999 - 2000	1.02	-0.15	-1.66	-0.55	0.59	-1.45	-0.66	-0.96	-0.95	-0.74	-0.19	-0.40
2000 - 2001	-2.23	-1.25	0.81	-1.08	-0.05	0.01	-0.88	-0.73	-0.77	0.53	-0.19	-0.42
2001 - 2002	0.48	1.04	0.55	-0.04	-1.50	0.99	1.84	0.37	0.48	0.05	-0.19	0.01
2002 - 2003	0.54	0.21	1.11	-0.35	0.95	1.65	1.03	-2.53	1.65	-0.93	1.34	0.54
2003 - 2004	-0.53	0.49	-0.92	-0.43	0.44	1.13	-1.22	0.80	-0.98	-0.16	-0.19	-0.67
2004 - 2005	0.28	1.44	0.23	0.17	-0.09	0.65	0.22	-0.36	0.03	-0.68	0.48	0.19
2005 - 2006	-0.09	-0.50	0.00	2.47	0.73	-0.02	-0.50	-0.37	-1.63	1.65	0.29	-0.20
2006 - 2007	0.25	0.19	0.34	-0.98	-1.19	-0.66	1.27	-0.77	-0.28	1.96	0.78	-0.67
2007 - 2008	-1.52	0.87	0.33	-1.56	0.08	-0.90	-1.37	-0.18	-1.16	0.92	0.74	1.44
2008 - 2009	0.49	-0.88	0.08	0.63	0.78	1.33	-0.15	-0.54	0.81	1.59	0.31	1.47
2009 - 2010	-0.91	1.22	0.02	1.04	1.42	-0.45	-0.19	0.83	1.35	-0.93	0.08	-0.19
2010 - 2011	0.22	-1.96	-1.35	-1.37	-0.12	-0.95	2.48	0.63	0.85	0.92	-0.19	1.64
2011 - 2012	1.73	-1.00	-1.10	1.17	0.58	1.43	-0.40	-0.25	-0.31	0.03	0.17	0.27
2012 - 2013	0.05	-0.65	0.80	1.59	0.47	-0.39	-0.69	0.16	-0.32	-0.93	-0.19	0.16
2013 - 2014	-0.63	-0.25	-0.03	-0.26	2.56	-0.21	0.63	-1.13	0.64	0.92	0.59	0.90
2014 - 2015	0.91	0.68	0.52	0.79	-1.35	0.60	0.06	0.82	-0.74	-0.68	2.34	-0.35
2015 - 2016	1.55	0.15	0.24	0.01	-2.01	0.70	-0.78	0.72	1.37	-0.20	-0.19	0.92
2016 - 2017	1.15	-1.10	0.95	-0.95	-0.80	-0.68	0.40	1.38	-1.65	0.08	0.38	-0.67
2017 - 2018	-0.33	0.67	-0.55	0.38	-0.09	-0.52	-0.54	1.25	0.25	-0.93	-0.19	-0.64
2018 - 2019	0.86	-0.56	1.40	0.52	-0.19	-0.65	-0.11	-1.32	-0.44	-0.63	0.10	-0.52
2019 - 2020	-0.43	-0.67	-1.96	1.49	2.17	0.51	0.46	-0.66	-0.06	1.38	1.05	-0.07

Table 3.1. One month data table with colour-drought referring

### 3. Results

#### 3.1. One Month Data Results

One month of data was analyzed and results were shown in table 3.1. It was seen that there is severe drought calculated in April 1982-1983, October and January 1998-1999, October 2000-2001, May 2002-2003, and February 2015-2016. As seen in the data, there are no high drought risks that happened in any years of July August, and September months.

Unevenness of the precipitation can be seen in the whole data and each year. On the left scheme as seen there are also very humid months are seen especially at the end of the summer and the beginning of the spring. The main drought can be seen at the end of spring and the beginning of winter (October-

November-December). To compare years, the severity of the drought per month decreased between 2010-2020 compared to 1990-2010 years.

### 3.2. Three Months Data Results

Year	Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sep
1980 - 1981	-0.38	0.57	0.45	-1.03
1981 - 1982	0.01	-0.55	0.97	-0.08
1982 - 1983	0.43	-1.32	0.16	0.39
1983 - 1984	-0.30	-0.47	1.01	-0.90
1984 - 1985	0.05	2.20	-0.96	-1.44
1985 - 1986	-0.60	-1.24	0.57	-0.85
1986 - 1987	-0.55	0.83	-1.28	0.81
1987 - 1988	1.24	0.75	1.45	1.40
1988 - 1989	0.32	-0.42	-1.04	0.23
1989 - 1990	0.55	-0.53	0.32	-1.60
1990 - 1991	-0.84	-0.17	-0.17	-1.08
1991 - 1992	1.00	-0.23	1.27	0.89
1992 - 1993	0.22	-0.76	1.91	-0.81
1993 - 1994	1.32	-0.95	1.41	0.64
1994 - 1995	2.06	-1.34	0.07	1.98
1995 - 1996	-1.38	0.54	-1.21	0.94
1996 - 1997	0.07	0.20	-1.02	0.91
1997 - 1998	0.09	-0.47	-0.78	-1.16
1998 - 1999	-1.61	-0.96	-0.48	0.18
1999 - 2000	-0.16	-0.57	-1.61	-1.23
2000 - 2001	-1.45	-0.58	-1.56	-0.43
2001 - 2002	1.12	-0.20	1.43	-0.35
2002 - 2003	0.97	1.15	0.45	0.63
2003 - 2004	-0.85	0.52	-0.47	-1.21
2004 - 2005	1.14	0.20	-0.31	-0.10
2005 - 2006	-0.82	1.53	-1.20	0.51
2006 - 2007	0.16	-1.49	0.17	0.73
2007 - 2008	-0.14	-1.09	-1.50	1.36
2008 - 2009	-0.43	1.21	-0.27	1.49
2009 - 2010	0.19	1.09	0.93	-0.76
2010 - 2011	-2.28	-1.21	2.17	1.38
2011 - 2012	0.50	1.38	-0.77	-0.01
2012 - 2013	-0.15	0.75	-0.62	-0.40
2013 - 2014	-1.05	1.70	-0.13	0.90
2014 - 2015	1.17	0.02	0.24	0.88
2015 - 2016	1.33	-0.44	0.59	0.51
2016 - 2017	0.79	-1.32	0.80	-0.60
2017 - 2018	-0.37	-0.27	0.58	-2.33
2018 - 2019	1.08	-0.30	-1.25	-1.14
2019 - 2020	-2.41	2.19	-0.34	0.68

**Table 3.2.** Three months data table with colour-drought referring with data range of 1980-2020.

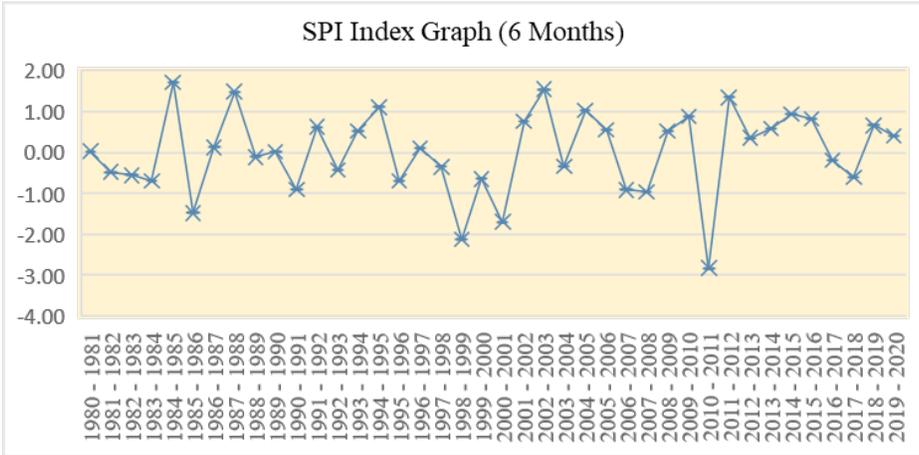
The values of the SPI index calculated according to the 3-month reference (October-December) are given below. It has been calculated as a moderately dry year in 1995-1996. Extreme drought was seen in 2010-2011. Although moderate seasonal droughts were observed in the mentioned months until 2019-2020, the driest year was seen this year (-2.41).

The values of the SPI index calculated according to the 3-month reference (January-March) are given below. It has been calculated as a moderately dry year in 1982-1983. 1984-1985 was the most humid of all years. The excess of snowfall in January-March generally prevented all data from being relatively dry. Except for the years 2006-2007 and 2016-2017, extreme drought was not observed in the whole data. The values of the SPI index calculated according to the 3-month reference (April-June) are given below. Moderate and extreme droughts were seen in 1984-1985, 1986-1987, and 1988-1989. Extremely dry years were in 2000-2001 and 2001-2002. There were no extremely dry years until 2020, and the humid year was 2010-2011.

The values of the SPI index calculated according to the 3-month reference (July-September) are given below. 1984-1985 was calculated as a moderately dry year. 1991 was moderately dry and 1989-1990 was extremely dry. It was calculated that it was extremely humid from 1994-1995. The highest extreme dry year was observed in 2017-2018. Other years have seen normal, mild, and moderate drought and precipitation.

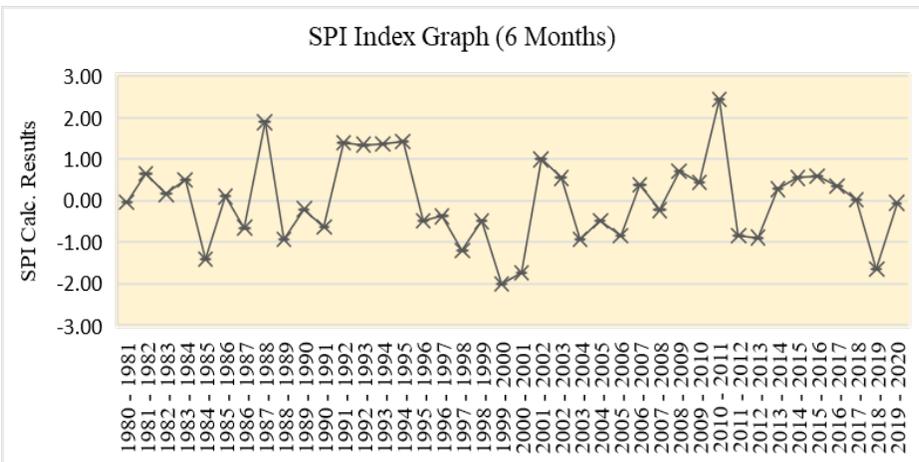
### ***3.3. Six Months Data Results***

The values of the SPI index calculated according to the 6-month reference data (October-March) are given below. 1984-1985 index value was 1.72 with extremely humid, between 1985-1986 moderate drought was seen with -1.49. Between 1987-1998 there was no extreme or severe drought. In 1998-1999, a severe drought was calculated. SPI, which was calculated to be extremely humid in 2003 the contrast entered its driest period in 2011, resulting in a severe drought. No drought has been observed until 2020 from this date (Figure 3.1.).



**Figure 3.3.** Graphical analysis of SPI with (October-March) reference values

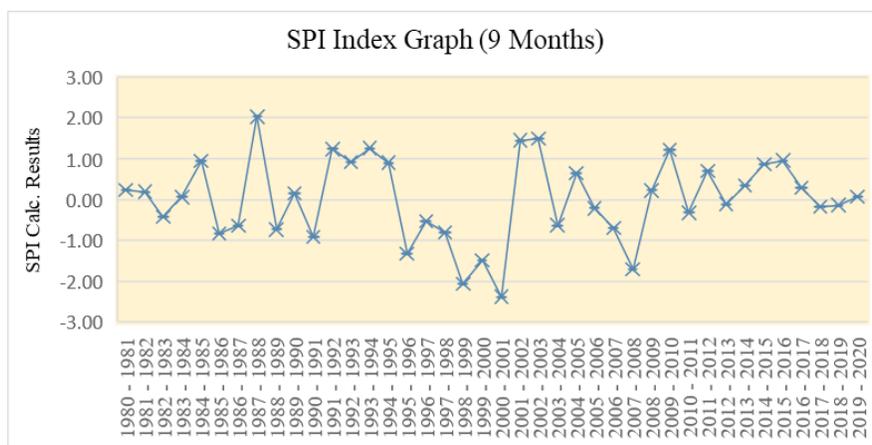
The values of the SPI index calculated according to the 6-month reference (April-September) are given below. 1984-1985 index value of -1.42 was moderately dry, and between 1987-1988 extremely humid season was observed with a 1.89 value. Between 1992 and 1996, it had a moderately humid year in contrast to drought in other years. In 1999-2000, a very extreme drought was calculated and it was the driest season in the 40-year data. 2018-2019 had an extremely dry season and was the driest season since the year 2000 (Figure 3.2.).



**Figure 3.4.** Graphical analysis of SPI with (April-September) reference values

### 3.4. Nine Months Data Results

In Figure 3.5, the data from October to June SPI were calculated according to the 9-month reference data. According to this 9-month calculation, SPI values of Van province between 1980 and 1986 were normal, moderately dry, and close to these index values. The value in 1988, was the highest in 40 years of data and shows that it is classified as extremely humid. From 1998 to 2001, extreme and severe dry years were experienced. SPI indices for 2002-2003 calculated these years as moderate humidity. From this year to 2020, except for 2007-2008 (extremely dry), values varied between normal and moderately dry/humid values.



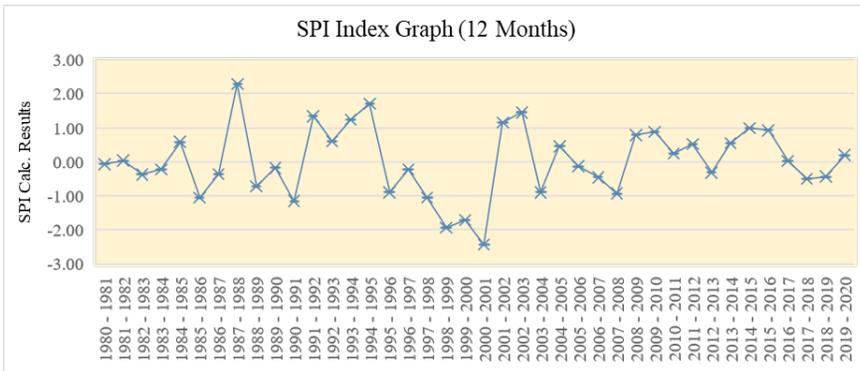
**Figure 3.5.** Graphical analysis of SPI with 9-month reference values

In Figure 3.5, the data from October to June SPI were calculated according to the 9-month reference data. According to this 9-month calculation, SPI values of Van province between 1980 and 1986 were normal, moderately dry, and close to these index values. The value in 1988 was the highest in 40 years of data and shows that it is classified as extremely humid. From 1998 to 2001, extreme and severe dry years were experienced. SPI indices for 2002-2003 calculated these years as moderate humidity. From this year to 2020, except for 2007-2008 (extremely dry), values varied between normal and moderately dry/humid values.

### 3.5. Annual Data Results

When we look at the 12-month reference values according to the SPI index, it is seen that there were different values of drought data according to the years.

With the effect of precipitation irregularity, continuously variable results have emerged over 40 years. SPI values of Van province between 1980 and 1985 are between normal and close to normal values. Between 1985 and 1986, moderate drought was observed. Between 1987 and 1988, contrary to this drought, it had an extremely humid year (2.29). This value, which is above 2.00 according to the SPI value, is the highest in 40 years of data. After this year, normal, moderately dry, and moderately humid years continued until 1998, except 1995. The years 1999, 2000, and 2001 were calculated as extreme and severe drought years. The values have changed between normal and slightly dry-light humid values. Extreme drought or humid values were not observed.



**Figure 3.6.** Graphical analysis of SPI with 12-month reference values

#### 4. Conclusion and Discussion

Drought analysis of Van province, located in the Eastern part of Turkey, was investigated by using a meteorology station (17172) display data between 1980 to 2020 years by using Standardize Precipitation Index with month, 3-month, 6 months, 9 months and 12-month data that ignites periodically through using DrinC software.

Drought at some years and months of the classifying ranges were found to be comparable to each other. According to the results obtained, 1-month SPI index values of Van station in from July to September months there were no drought risks in these months for the whole data. However, from time to time, it is seen that there are severe drought periods, especially in October occurred in very several years like 1998-1999 and 2001-2002. But for the whole data there was an unregular drought calculated which shows for each year there is this risk of irregularity that can cause drought in any month except mentioned months. For 3 months, the drought risks were screened for whole seasons and

years. After the 2010 year, there were severe drought seasons such as in the October-December period in 2010-2011 and 2019-2020. Concordantly, the number of humid 3 months data before 2010 was more than the data after this year. The risk of drought for 9 months of data (October-June) shows that there was just one year as a risk was observed in 2000-2001. The risk and irregularity of the drought are not seen after 2011-2012 showing that from October to July there can be drought risk with the not severe level expected. As a result of the calculated 12-month indices values, drought around extreme and moderate was experienced in years between 1997-2001. According to the 40-year data used in the study, it has been determined that the drought around normal is constantly seen in the field, and extreme arid and severe drought classes are experienced from time to time. This is an indication of the importance of water use in the region, especially in agricultural areas. When the drought frequency of the drought SPI values obtained from Van meteorology station data is examined, it is observed that there has been an increase in 3-month winter data in the last ten years, that is, the drought experienced in the winter months has increased in recent years.

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