Online ISSN : 2249-4626
Print ISSN : 0975-5896
DOI : 10.17406/GJSFR

Global Journal

of Science Frontier Research: D

Agriculture & Veterinary

Awareness of Mango Farmers

Efficacy of Secondary Metabolites

Highlights

Rates of Nitrogen Fertilizer

Inhibition of Zymoseptoria Tritici

Discovering Thoughts, Inventing Future

VOLUME 20 ISSUE 1 VERSION 1.0

Global Journal of Science Frontier Research: D
Agriculture & Veterinary

Global Journal of Science Frontier Research: D
Agriculture & Veterinary
Volume 20 Issue 1 (Ver. 1.0)

Open Association of Research Society

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Offset Typesetting

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Global Journal of Science Frontier Research: D
Agriculture and Veterinary
Volume 20 Issue 1 Version 1.0 Year 2020
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Yield Stability in Forage Maize across Selected Test-Environments

By S. H. Mohammed & M. I. Mohammed

Abstract- Assessing new maize cultivars requires studying both yield and stability performance across the major range of environments. Four trials were conducted in Sudan (Africa) during 2013 – 2014. Nine maize genotypes were investigated for forage yield stability across 8 test-environments created by a combination of 2 levels of location, season and watering regime assumed to impose respective effects of salt, heat and water stresses. Wricke's ecovalence, Eberhart-Russell and AMMI stability models were employed to study yield stability. The genotypes and watering regimes were arranged in RCB design in split-plot experiment. The study revealed maize hybrids having broad and specific responses to the studied environments with most genotypes showing consistent stability performance in the three models. Two of the 3 top-yielding hybrids showed relative stability whereas the third one exhibited specific adaptability to low yielding environments. It was concluded that yield stability could be better investigated if the varieties are purposely subjected to major factors affecting yield in a given domain. Different stability models were recommended to avoid limitations arising from using a single model.

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GJSFR-D Classification: *FOR Code: 820401, 070199*

Strictly as per the compliance and regulations of:

Yield Stability in Forage Maize across Selected Test-Environments

S. H. Mohammed [α] & M. I. Mohammed [σ]

Abstract- Assessing new maize cultivars requires studying both yield and stability performance across the major range of environments. Four trials were conducted in Sudan (Africa) during 2013 – 2014. Nine maize genotypes were investigated for forage yield stability across 8 test-environments created by a combination of 2 levels of location, season and watering regime assumed to impose respective effects of salt, heat and water stresses. Wricke's ecovalence, Eberhart-Russell and AMMI stability models were employed to study yield stability. The genotypes and watering regimes were arranged in RCB design in split-plot experiment. The study revealed maize hybrids having broad and specific responses to the studied environments with most genotypes showing consistent stability performance in the three models. Two of the 3 top-yielding hybrids showed relative stability whereas the third one exhibited specific adaptability to low yielding environments. It was concluded that yield stability could be better investigated if the varieties are purposely subjected to major factors affecting yield in a given domain. Different stability models were recommended to avoid limitations arising from using a single model.

Keywords: wricke's ecovalence, eberhart and russell, AMMI, GxE.

I. Introduction

Maize (*Zea mays* L.) is one of the World's three most important cereal crops. It is the primary source for coarse-grain representing 55% of the World consumption of animal feed [1]. Although the crop is cultivated in a wide range of environments due to its relatively wide adaptability [2] it is the least tolerant to abiotic stresses among cereals. Drought, salinity and elevated temperatures coupled with low humidity [1] are among the major abiotic stresses that negatively impact maize production.

Identification of high yielding cultivars with wide adaptability is the ultimate aim of plant breeders. However, attaining this goal is complicated by the genotype x environment (GxE) interaction. Therefore, assessing of new cultivars must be based not only on their yielding ability but also on their stability and adaptability across broad range of environments to avoid the misleading results caused by GxE interaction and to identify cultivars having the adaptability to specific environments. Several models could be used to study GxE interaction. The Wricke's ecovalence model [3] simply quantify the contribution of each genotype in GxE interaction as a measure of stability related directly to the non-additive structure. Joint linear regression is another widely used model in plant breeding for analyzing and interpreting GxE interaction and determining yield stability of genotypes. It involves the regression of genotype means on an environmental index [4] and provides means of testing whether the genotypes have characteristic linear responses to environmental change [5]. Additive main effects and multiplicative interaction (AMMI) model is a powerful tool in diagnosing GxE patterns of interaction [6]. It is a multimodal approach that proved useful in understanding complex genotype x environment interactions.

The objectives of this study were to investigate forage yield stability of maize hybrids subjected to predetermined test-environments reflecting various levels of abiotic stress.

II. Materials and Methods

The experiment was conducted in Khartoum State during 2013-2014 under two seasons (summer and winter) and two locations: Shambat (Lat. 15° 39' N; Long. 32° 31' E; Alt 380 masl) and Soba (Lat.15° 24' N; Long.32° 32' E; Alt 380 masl). In each location the trial was carried out in the Experimental Farm of the Agricultural Research Corporation (ARC).

a) Soil and climatic conditions

The soil at Shambat is well-drained loamy clay, non-saline and non-sodic, with pH ranging from 7.71 to 7.91. The soil at Soba is hazarded by salinity (ECe = 12 - 14 dS/m) and sodality (ESP = 24 - 27, SAR = 16 –23) with high clay content, low infiltration and permeability, low organic matter, low nitrogen and high pH. The average min-max temperature during the winter season (Nov. –Feb.) ranged 15-20°C and 32-38°C whereas that at summer (April-July) ranged 25.0-28.4°C and 36.9-42.0 °C. The weather is dry in both growing seasons especially during winter. For further details of soil and climatic conditions see Appendices I through V.

b) The plant material

The plant materials used in the study (Table 1) included nine maize genotypes comprising 8 hybrids plus one open-pollinated cultivar. Six of the maize genotypes have already been released for commercial production in Sudan.

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Table 1: Plant material used in the study

Genotypes	Type/Color	Source
PAN6966	Yellow maize hybrid	Pannar Co. South Africa
PAN-12	Yellow maize hybrid	Pannar Co. South Africa
PAN-14	Yellow maize hybrid	Pannar Co. South Africa
PAN6P-110	Yellow maize hybrid	Pannar Co. South Africa
Hytech1100	White maize Hybrid	MisrHytech Co. Egypt
Hytech2066	Yellow Maize hybrid	MisrHytech Co. Egypt
Hytech2031	White Maize hybrid	MisrHytech Co. Egypt
Hytech2055	Yellow Maize hybrid	MisrHytech Co. Egypt
Hudieba2	Yellow Maize (open pollinated)	Agric. Res. Corporation (ARC) Sudan

c) Cultural practices

Four trials were conducted in the winter and summer seasons at Shambat and Soba locations. Unless otherwise indicated, the cultural practices followed were the same in the different trials. The land was disc ploughed, disc harrowed and leveled by the scraper to obtain fine seed bed. Ridging was done at 0.75 m spacing. The plot consisted of four ridges 4 m long. Two seeds were placed in holes spaced at 10 cm on one side of the ridge. The winter sowing was on the 8th and 12th of Dec. 2013 in Soba and Shambat, respectively. The summer sowing was on the 13th and 19th of May 2014 in Soba and Shambat, respectively. Nitrogen fertilizer (55 kg N/ha) was applied at growth stage-2 (four leaves completely unfolded). Weed population was controlled by hand weeding.

d) Treatments and the experimental design

The genotypes were subjected to the following main treatments factors in a randomized complete block design (RCBD) with three replications:

- Two watering intervals applied at one and two weeks using split-plot experiment with watering regimes assigned to the main plots and the genotypes to the sub-plots
- Two growing seasons: Summer and winter (normal).
- Two locations: Soba and Shambat.

The combination of location, season and watering regime (2x2x2) provided 8 test-environments (Table 2) assumed to bring about different test environments used to investigate yield stability of the 8 maize genotypes

Table 2: The test-environments

S. No.	Location	Season	Year
1	Soba	Winter	13/2014
2	Shambat	Winter	13/2014
3	Soba	Summer	2014
4	Shambat	Summer	2014
5	Soba	Summer	2017
6	Shambat	Summer	2017
7	Soba	Winter	2017/18
8	Shambat	Winter	2017/18

III. Data Collection and Statistical Analysis

Forage yield was estimated at the milk stage from the two inner rows of each plot leaving 0.5 m from each side of the ridge. The plants were cut at the ground level and weighed immediately using spring balance. Dry matter yield (DMY, t/ha) was estimated from a random sample of 0.5 kg taken from the fresh harvested plants in each plot and air-dried to a constant weight. Days to 50% tasselling, plant height, stem diameter and quality traits (NDF, ADF, CP) were studied but will not be highlighted in this study.

Analysis of variance was performed following the standard procedure of analyzing split plot in RCB design [7]. Combined analysis of variance to assess the magnitude of genotype-environment interaction (GEI) was performed. Then mean squares of GEI was used to test the effect of genotypes. Analysis of yield stability for nine maize genotypes was carried out over the eight environments using the following stability models:

a) Wricke's ecovalence (Wi)

According to this model, the stability of the genotype is its interaction with environments, squared and summed across environments [3]. The formula of this model is as follows:

$$Wi = \sum(Y_{ij} - Y_{\cdot j} - Y_i + Y\ldots) \qquad [2]$$

Where: Y_{ij} = Mean of genotype i in environment j, $Y_{\cdot j}$ = Mean yield of genotype across environments, Y_i = environment mean, Y… = Overall mean.

b) Eberhart and Russell Stability Regression Model

The equation underlying this model [5] is as follows:

$$Y_{ij} = m + B_i I_j + \delta_{ij}$$

Where:
i=1, 2,…..g (number of genotypes)
j=1, 2,…...s (number of environment)
Y_{ij}= The mean yield of i^{th} genotype in the j^{th} environment.
m = The mean of all genotypes overall environments
B_i = The regression coefficient of the i^{th} genotype on environment index, which measures the response of this genotype to varying environments.

I_j= The environment index which is defined as the deviation of the mean of all genotypes at a given environment from the overall mean.
δ_{ij} = The deviation from regression of i^{th}genotype at j^{th} environment.

The regression coefficient (bi), was estimated as:

$$bi = j\sum Y_{ij}Ij / \sum I^2j$$

Where:
bi = regression coefficient of the i^{th} genotype
Y_{ij} =the mean yield of i^{th} genotype in the j^{th} environment.
Ij= environmental index obtained as the mean of all genotypes at the j^{th} environment minus the grand mean.

Deviation from regression (σ^2d) suggested by Eberhart and Russel, (1966) estimated as:

$$\sigma^2d = \sum\delta^2_{ij}/(S-2)\text{-}Se^2/r,$$

Where:
$\delta^2_{ij} = (\sum Y_{ij} - Yi/g) - (\sum Y_{ij} I^2)$
r = number of replications
g = number of genotypes, and s=number of environment.
Se = the pooled error.
δ_{ij} = the deviation from regression of i^{th} genotype at j^{th} environment.
Y_{ij} =the mean yield of i^{th} genotype in the j^{th} environment.

c) Additive Main Effects and Multiplication Interaction (AMMI) Stability Model

The AMMI model equation [8] is:

$$Y_{ij} = \mu + Gi + Ej + \sum_{\kappa=1}^{n} \lambda\kappa\alpha_{ik}\gamma_{jk} + e_{ij}$$

Where:
Y_{ij} is the yield of the i^{th} genotype in the j^{th} environment;
μ is the grand mean;
Gi and Ej are the genotype and environment deviations from the grand mean, respectively;
$\lambda\kappa$ is the eigen value of the PCA analysis axis ;
κ; α_{ik} and γ_{jk} are the genotype and environment principal component scores for axis ;
κ; n is the number of principal components retained in the model
e_{ij} the residual.

The statistical package Agrobase [9] was used to run the three models of stability analysis

IV. Results

Mean squares from combined analysis of variance for forage yield of 9 maize genotypes over the 8 test-environments are presented in Table 3. Differences among environments, genotypes and genotype by environment interaction were highly significant for forage yield.

Table 3: Mean squares from combined ANOVA for forage yield of nine maize genotypes studied over eight environments

Source of variation	DF	Dry matter yield (t/ha)
Environments (E)	7	178.137**
Reps within (E)	16	0.714
Genotypes (G)	8	31.031**
G×E	56	3.293**
Residual	128	0.472

** = Highly significant at 0.01 probability level

a) Wricke Ecovalence

Wi-ecovalance stability values and mean performance of nine maize genotypes across eight environments for DMY are presented in Table 4. The genotype Hytech2055 ranked top in forage yield (10.8 t/ha) coupled with the second-lowest stability value (wi = 2.961). PAN12 ranked third in both yield (10.3 t/ha) and stability value (wi = 3.475). PAN14 exhibited the lowest stability value (wi =2.517), coupled with the lowest forage yield (7.75 t/ha). In contrast, Hytech2031 averaged the second-top yield (10.5 t/ha) coupled with the second-highest stability value (wi = 9.850).

Table 4: Stability values (Wi-ecovalance) and mean performance in dry matter yield (DMY) of maize genotype

Genotypes	DMY (t/ha)	Wi-ecovalance†	Variations explained (%)
PAN6966	8.38 (6)	5.253 (5)	8.55
PAN12	10.3 (3)	3.475 (3)	5.65
PAN14	7.75 (9)	2.517 (1)	4.09
PAN6P-110	8.50 (5)	8.739 (7)	14.22
Hytech1100	8.13 (8)	17.531 (9)	28.52
Hytech2066	9.50 (4)	6.961 (6)	11.32
Hytech2031	10.5 (2)	9.850 (8)	16.02
Hytech2055	10.8 (1)	2.961 (2)	4.82
Hudeiba2	8.38 (7)	4.184 (4)	6.81
Grand mean	9.13		

Figures between brackets denote rank
† : Smaller value indicates better yield stability

b) Eberhart and Russell's Stability Model

Table 5 shows the ANOVA from Eberhart-Russell Regression Model for forage yield of nine maize genotypes tested across 8 environments. The analysis of variance revealed significant differences among genotypes for forage yield. The GxE (linear) was significant. Table 6 shows the parameters of yield stability for DMY of nine maize genotypes across 8 environments. The genotype Hytech2055 ranked top in

forage yield (10.8 t/ha), showed the closest regression coefficient to unity (bi=1.0309) and small deviation from regression (σ^2d=0.320). PAN12 ranked third in forage yield (10.3 t/ha), showed regression coefficient close to unity (bi=1.0736) and small deviation from regression (σ^2d = 0.211). Hytech2031ranked second in forage yield (10.5 t/ha) with regression coefficient well below unity (bi= 0.7993) and exhibited the second largest deviation from regression (σ^2d = 1.165). Hytech2066 showed above average yield, regression coefficient ranking second in closeness to unity and large deviation from regression.

Table 5: ANOVA from Eberthart and Russell's stability model for dry matter yield (t/ha) of nine maize genotypes

Source	DF	MS
Genotypes (G)	8	10.344**
Environment (E).+ in G.x E.	63	7.573
E. in linear	1	0.000
G x E. (linear)	8	1.748*
Pooled deviation	54	0.879
Residual	144	0.166

*, ** = Significant and highly significant at 0.05 and 0.01 probability level, respectively

Table 6: Mean performance and stability parameter of maize genotypes evaluated across eight environments using Eberthart and Russell's stability model

Genotypes	Dry matter yield (t/ha)		Regression coefficient (bi)		Deviation from linearity of regression (σ^2d)	
PAN6966	8.38	(6)	1.1855	(5)	0.521	(5)
PAN12	10.3	(3)	1.0736	(3)	0.211	(2)
PAN14	7.75	(9)	1.1563	(4)	0.266	(3)
PAN6P-110	8.50	(5)	0.7085	(9)	0.636	(6)
Hytech1100	8.13	(8)	1.2724	(8)	2.184	(9)
Hytech2066	9.50	(4)	0.9660	(2)	0.985	(7)
Hytech2031	10.5	(2)	0.7993	(7)	1.165	(8)
Hytech2055	10.8	(1)	1.0309	(1)	0.320	(4)
Hudeiba2	8.38	(7)	0.8075	(6)	0.128	(1)
Grand mean	9.13					

Figures between brackets denote rank

c) AMMI Stability model

The mean squares from AMMI analysis of variance (Table 7) indicated significant variations among the genotypes, the environments and their interaction for forage yield. The GxE is highly significant accounting for 10.53% of the sum of squares. The genotype x environment interaction (GxE) was partitioned into seven interaction principal component analysis axis (IPCA). The IPCA1 and IPCA2 scores are highly significant explaining 51.79% and 22.27% of the variability relating to GxE, respectively (totaling 74.1%). Table 8 shows the IPCA axis scores and forage yield for nine maize genotypes averaged across 8 environments. Hytech-2055, the highest yielding genotype scored the second lowest value in IPCA1 (0.2686) and the lowest value in IPCA2 (0.3191). The genotype PAN12 that ranked third in forage yield scored the lowest value in IPCA1 (-0.0726) coupled with high value in IPCA2 (-0.7807). Hytech2031, the second highest yielding genotype scored the second highest value in IPCA1 (0.9609) and IPCA2 (0.8561).

Table 7: Mean squares from AMMI stability model and the percentage of G x E explained by each IPCA† for dry matter yield (t/ha) of nine maize genotypes grown in eight environments

Source	DF	SS	MS	F-value	Prob.> F	Variations explained (%)
Total	215	1751.472				100
Environments (E)	7	1246.958	178.137 **	249.60	0.0000	71.2
Reps within E	16	11.419	0.714			0.65
Genotypes (G)	8	248.250	31.031 **	9.42	0.0000	14.17
G × E	56	184.417	3.293 **	6.98	0.0000	10.53
IPCA1	14	95.513	6.822	14.45	0.0000	(51.79)
IPCA2	12	41.076	3.423	7.25	0.0000	(22.27)
IPCA3	10	25.605	2.560	5.42	0.0000	(13.88)
IPCA4	8	13.509	1.689	3.58	0.0009	(7.33)
IPCA5	6	6.873	1.145	2.43	0.0296	(3.73)

IPCA6	4	1.794	0.448	0.95	0.4376	(0.97)
IPCA7	2	0.048	0.024	0.03	0.9502	(0.03)
Residual	128	60.427	0.472			3.45

†: IPCA = Interaction principal component analysis axis.
Figures between brackets denote percentage explained by IPCAs from that explained by GxE (10.53)
** = Highly significant at 0.01 probability level

Table 8: IPCA† scores and mean performance in dry matter yield (DMY) of nine maize genotype

Genotypes	DMY (t/ha)		IPCA1		IPCA2
PAN6966	8.38	(6)	-0.4460	(4)	-0.9833
PAN12	10.3	(3)	-0.0726	(1)	-0.7807
PAN14	7.75	(9)	0.3278	(3)	-0.5303
PAN6P-110	8.50	(5)	0.8789	(7)	-0.4147
Hytech1100	8.13	(8)	-1.6497	(9)	0.5641
Hytech2066	9.50	(4)	-0.7686	(6)	0.3310
Hytech2031	10.5	(2)	0.9609	(8)	0.8561
Hytech2055	10.8	(1)	0.2686	(2)	0.3191
Hudeiba2	8.38	(7)	0.5007	(5)	0.6388
Grand mean	9.13				

†: IPCA = Interaction principal component analysis axis
Figures between brackets denote rank

d) Comparison of yield stability ranking in the different models

Table 9 shows forage yield and stability ranking in 3 stability models for nine maize genotypes. As could be noticed in this table there were no major changes in stability ranking for the 9 maize genotypes across the 3 stability model. Hudieba2 might be one of the exceptions ranking first in Eberhart and Russel's model, fourth and fifth in Ecovalance and AMMI models, respectively. PAN12, the third-highest yielding genotype averaged the lowest rank across the 3 stability models. Hytech2055, the highest yielding genotype ranked third in average stability ranking. Hytech2031, the second-highest yielding genotype averaged the second highest stability rank across the 3 models.

Table 9: Dry matter yield (DMY) and average stability ranking of maize genotypes tested across eight environments

Genotypes	DMY(t/ha)		Wricke (wi)-ecovalance		Eberhart & Russel's (deviation σ^2d)		AMMI (IPCA1) scores		Average stability rank
PAN6966	8.38	(6)	5.253	(5)	0.521	(5)	-0.4460	(4)	4.7
PAN12	10.3	(3)	3.475	(3)	0.211	(2)	-0.0726	(1)	2
PAN14	7.75	(9)	2.517	(1)	0.266	(3)	0.3278	(3)	2.3
PAN6P-110	8.50	(5)	8.739	(7)	0.636	(6)	0.8789	(7)	6.7
Hytech1100	8.13	(8)	17.531	(9)	2.184	(9)	-1.6497	(9)	9
Hytech2066	9.50	(4)	6.961	(6)	0.985	(7)	-0.7686	(6)	6.3
Hytech2031	10.5	(2)	9.850	(8)	1.165	(8)	0.9609	(8)	8
Hytech2055	10.8	(1)	2.961	(2)	0.320	(4)	0.2686	(2)	2.7
Hudeiba2	8.38	(7)	4.184	(4)	0.128	(1)	0.5007	(5)	3.3
Grand mean	9.13								

Figures between brackets denote rank

V. Discussion

The highly significant genotype x environment interaction (GxE) validates the performing of stability analysis to know the contribution of each genotype to GxE which is the basic cause for differences between genotypes in their yield stability [10]. In the present study, the maize genotypes were studied under eight environment representing stress conditions resulting from the main effects of heat, salt, water and their interactions. Thus, the assessment of genotypes for yield stability should be considered within the context of the studied environments. We think that the test environments used in this study are appropriate since maize was evaluated as a forage crop assumed to have less demands of input and capable to flourish under marginal environments.

No one biometrical model can adequately explain the stability performance of genotype across environment [11]. In this study, three models with different statistical approaches were used to avoid limitations arising from using a single model. In Wricke's Ecovalence model the cultivars with the lowest value contributed the least to the GxE interaction and are therefore more stable. Based on yield level and Ecovalence value the hybrid Hytech2055 can be regarded as the most stable as it ranked top in forage yield with the second lowest Ecovalence value. Similar conclusions were reported regarding the grain yield stability of the hybrid Hytech2055 [12]. The hybrid PAN12 came second in yield stability ranking third in forage yield coupled with the third lowest Ecovalence value. Hytech2031, though ranked the second top in forage yield failed to demonstrate good yield stability showing the second largest Ecovalence value.

In Eberhart and Russell model [5], two statistics were employed, namely: the regression coefficient as a measure of response [4] and deviation from linearity of regression [5] as stability measure. Results based on this model and similar techniques may be misleading if the genotype response over environment is not linear [6]. However, in this study the linearity of GxE is highly significant, validating the results obtained from Eberhart and Russell's model. Mean yield of entries across all environments and regression coefficients are important indicators of cultivar adaptation [4]. A regression coefficient approximating 1.0 indicated average stability, and in association with high yield, the entry possesses general adaptability. However, entries with a low yield would be poorly adapted to the environment. Regression coefficient values increasing above 1.0 describe genotypes with increasing sensitivity to environmental change, thus below average stability. Regression coefficients decreasing below 1.0 provide a measure of greater resistance to environmental change, thus above average stability. However, regression coefficients must also be associated and interpreted with genotype mean yields to determine adaptability. In addition to the regression coefficient, Eberhart and Russell [5] added deviation from the regression as a measure of stability, where an entry would be considered stable with a deviation close to zero. Thus, based on the results of this study, the hybrid Hytech2055 exhibited the best general adaptability ranking top in forage yield with the least regression coefficient value. It showed moderate stability value ranking fourth in the deviation from the linearity of regression. The hybrids PAN12 and Hytech2066 came second in general adaptability, however, the former showed good stability parameter ranking the second-lowest in the deviation from linearity. The hybrid Hytech2031 though ranking second in forage yield, however, its regression coefficient value was well below unity suggesting greater resistance to environmental change, and therefore increasing specificity of adaptability to low-yielding environments. This was in conformity with the best yield obtained by this hybrid under full stress level. Therefore, Hytech2031 could have the relative advantage over the studied cultivars for forage production under the salt affected areas. Similar conclusions were reported for the adaptability of the hybrid Hytech2031to low-yielding environments [12].

The Additive Main effects and Multiplicative Interaction method (AMMI) employs the ANOVA procedure and Principle Component Analysis (PCA) to extract a new set of coordinate axes (IPCA) which account more effectively for the interaction patterns [13]. The more the IPCA scores approximate zero, the more stable the genotype is overall the environments sampled. Using PCA, the GxE was decomposed into 7 IPCAs two of them (IPCA1 and IPCA2) explained 74% of GxE variations into pattern-rich model. The variability relating to IPCA3 through IPCA5, though significant was small, therefore regarded as part of the residual. Based on the first two IPCAs, the hybrid Hytech2055 exhibited the best stability score followed by PAN12. The high yielding hybrid Hytech2031showed considerably high scores in both IPCAs pointing to its adaptability to specific environments. As previously discussed Hytech2031 showed specific adaptation to the low yielding environment based on the Eberhart and Russell's stability model. In fact, AMMI model is more powerful in detecting the environments to which genotypes are adapted by employing Biplot analysis [6], However, this feature of AMMI analysis was not used in this study.

The study revealed that there were no major differences between the results obtained from the stability models used in this study. The average rank of genotypes based on the 3 stability models was more or less similar to that obtained for each model. Such conformity gives more reliability to the results obtained.

VI. Summary and Conclusion

The study revealed maize hybrids having broad and specific responses to the studied environments. Yield stability could be better investigated if the varieties are purposely subjected to major factors known to affect yield in a given domain. We recommend using different stability models to avoid limitations arising from using a single model.

Conflict of Interest

The authors declare that there is no conflict of interest.

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Appendix I: Chemical and physical soil properties of the experimental site at Shambat

Depth (cm)	Chemical properties				Physical properties		
	pH	EC (dS/m)	Na (mmol+l)	SAR	Clay (%)	Silt (%)	Sand (%)
0-15	7.79	1.4	5.1	2.4	42.1	15.9	42.0
15-35	7.88	1.0	4.3	2.5	39.6	15.8	44.6
35-51	7.87	1.2	7.1	4.5	44.1	16.4	39.5
51-75	7.91	2. 0	12.5	6.3	51.4	16.6	32.0
75-120	7.71	2.2	16.0	9.2	50.0	16.6	33.4

Appendix II: Chemical soil properties of the experimental site at Soba

Depth	pH paste	pH 1:5	EC dS/m	SAR	ESP
0 - 30	8.1	8.8	14.0	23.0	27.0
30 - 60	8.3	8.9	12.0	16.0	24.0

Soluble Cations and Anions Saturation Extract (meq/L)

	Na	Ca	Mg	Cl	CaCo3	HCo3
0 - 30	10.3	32.5	6.0	8.3	0.0	4.6
30 - 60	19.0	32.5	6.5	6.3	0.0	4.3

Exchangeable Bases (Meq/100g)

	Na	K	CEC	N(%)	C/N%	Available P (ppm)
0 - 30	10.94	0.94	40	0.421	0.037	5.0
30 - 60	6.83	1.04	28	0.468	0.042	3.8

Source: Soil survey and land evaluation report. Land and Water Research Centre.ARC. Wad Medani. Sudan.

Appendix III: Physical soil properties of the experimental site at Soba

Depth (cm)	Mechanical analysis				Soil moisture				H_2o (Cm/cm)	
	Cs	Fs	Si	C	½ bar	15 bar	AWC	Vol%	Soil	Horizon
0 -20	8	18	37	37	27.2	13.6	13.6	22.0	0.33	6.6
20-50	4	30	21	45	28.9	15.5	13.4	21.8	0.22	6.6
50-80	7	17	33	43	28.5	15.3	13.2	22.8	0.23	6.9
80-120	4	23	33	40	27.1	14.6	12.5	20.8	0.21	8.4
120-160	5	20	29	46	36.1	19.0	17.1	30.4	0.30	12.0

Source: Soil survey and land evaluation report. Land and Water Research Centre.ARC. Wad Medani. Sudan.

Appendix IV: Monthly mean temperature (°C), rainfall and relative humidity (R.H %) during the winter season (2013/ 2014).

Month	Mean Temperature		R.H. (%)	Total rain fall (mm)
	Max.	Min.		
November 2013	34.0	20.0	27	0.0
December	32.0	16.0	32	0.0
January 2014	32.0	15.0	35	0.0
February	33.0	16.0	27	0.0
March	38.0	20.0	23	0.0

Source: Meteorological Authority, Ministry of environment Forestry and Physical Development (2014) Khartoum. Sudan.

Appendix V: Monthly mean temperature (°C), rainfall and relative humidity (R.H %) during the summer season (2014).

Month	Mean Temperature		R.H. (%)	Total rain fall (mm)
	Max.	Min.		
April	40.9	27.4	16	Trace
May	41.0	28.4	17	4.6
June	42.0	25.0	21	0.6
July	36.9	26.1	45	73.6

Source: Meteorological Authority, Ministry of environment Forestry and Physical Development (2014) Khartoum. Sudan.

Global Journal of Science Frontier Research: D
Agriculture and Veterinary
Volume 20 Issue 1 Version 1.0 Year 2020
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Efficacy of Secondary Metabolites Produced by *Bacillus Amyloliquefaciens* on the Inhibition of *Zymoseptoria Tritici*

By Barakat I., Chtaina N., Grappin P., El Guilli M., Abdenbi E. & Ezzahiri B.

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Abstract- The strain I3 of *Bacillus amyloliquefaciens*, which was isolated from soft wheat leaves, has revealed, *in vitro* and *in vivo,* a high antagonistic potential against septoria leaf blotch of wheat. In order to investigate the existence of antifungal molecules secreted by strain I3, the filtrates of this strain were tested for their inhibitory activity on the germination of pycnidiospores of the two strains of *Zymoseptoria tritici,* G1-1 and A5-1 isolated from soft wheat and durum wheat, respectively. The antibiosis assays showed a high level of inhibitory activity, with inhibition rates ranging from 94% to 99% compared to the control after 96 hours of incubation. Filtrate analysis by high-performance liquid chromatography coupled to mass spectrometry (HPLC-MS), have identified several families of lipopeptides reported as antifungal molecules (iturins, fengycins, and surfactins); and polyketides (macrolactins, chlorotetaines, and bacillaenes) which would also be responsible for the antagonistic activity against *Z. tritici*.

Keywords: nephelometry, antibiosis, HPLC-MS, secondary metabolites, bacillus amyloliquefaciens I3, zymoseptoria tritici.

GJSFR-D Classification: FOR Code: 070199

Strictly as per the compliance and regulations of:

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Barakat I. [α], Chtaina N. [σ], Grappin P. [ρ], El Guilli M. [ω], Abdenbi E. [¥] & Ezzahiri B. [§]

Abstract- The strain I3 of *Bacillus amyloliquefaciens*, which was isolated from soft wheat leaves, has revealed, *in vitro* and *in vivo,* a high antagonistic potential against septoria leaf blotch of wheat. In order to investigate the existence of antifungal molecules secreted by strain I3, the filtrates of this strain were tested for their inhibitory activity on the germination of pycnidiospores of the two strains of *Zymoseptoria tritici,* G1-1 and A5-1 isolated from soft wheat and durum wheat, respectively. The antibiosis assays showed a high level of inhibitory activity, with inhibition rates ranging from 94% to 99% compared to the control after 96 hours of incubation. Filtrate analysis by high-performance liquid chromatography coupled to mass spectrometry (HPLC-MS), have identified several families of lipopeptides reported as antifungal molecules (iturins, fengycins, and surfactins); and polyketides (macrolactins, chlorotetaines, and bacillaenes) which would also be responsible for the antagonistic activity against *Z. tritici*. A solid-liquid extraction method of these secondary metabolites from the confrontation zones between Bacillus and pathogenic strains, identified the same families of lipopeptides and polyketides with a higher relative abundance compared to the filtrates of the liquid-liquid extraction process.

Keywords: nephelometry, antibiosis, HPLC-MS, secondary metabolites, bacillus amyloliquefaciens I3, zymoseptoria tritici.

I. Introduction

Plant protection by Bacillus strains against pathogenic organisms are based on several modes of action, including the antibiosis mechanism, which is dependent on the production of different secondary metabolites that have a toxic effect against the pathogenic organisms. This mechanism is the most widely known and may be the most important mechanism used by Bacillus as plant growth promoting rhizobacteria to reduce the pathogen's infestation in the host plant's tissues [1]. The mechanism of antibiosis is direct inhibition of pathogen growth through the production of metabolites with antimicrobial properties [2,3]. Some Bacillus species, such as *Bacillus amyloliquefaciens*, can use up to 8.5% of their genetic material to synthesize a wide range of antimicrobial compounds, including lytic enzymes, antibiotics, polyketides, and a range of lipopeptides synthesized by non-ribosomal mechanisms [4,5]. The cyclic lipopeptides (surfactins, iturins and fengycins), are particularly interesting by the fact of being secreted at bio-effective levels in the natural conditions of the rhizosphere [6,7,8]. Also, bacillaenes, macrolactines, and chlorotetaines are polyketides with a high range of antibacterial and antifungal activities [4,9]. This antibiotic arsenal and the high ability to colonize roots probably explain the high biocontrol potential of the Bacillus genus *in vitro* and under natural conditions, [10,11,12,13].

In this study, we evaluated by nephelometry the effect of three filtrates - prepared with *B. amyloliquefaciens* I3 cultured alone or in the presence of the strain A5-1 of *Z. tritici* isolated from soft wheat and the strain G1-1 of *Z. tritici* isolated from durum wheat) - on the inhibition of pycnidiospores germination of septoria leaf blotch pathogen. Furthermore, the inhibiting/stimulating effects of the G1-1 and A5-1 strains on the secondary metabolite production in liquid and agar media was evaluated. HPLC-MS analyses of the secondary metabolites produced in *B. amyloliquefaciens* I3 filtrates identified cyclic lipopeptides and polyketides that could document the antagonistic activity.

II. Materials and Methods

a) Culture of Bacillus amyloliquefaciens I3

The bacterium was isolated from soft wheat leaves in a field located in the northern part of Morocco and exhibited high antagonistic activity against *Z. tritici* [14,15,16]. The identity of the bacterium was confirmed in the Laboratory of Exact and Natural Sciences at the University of Reims-France.

b) Antifungal activity of Bacillus amyloliquefaciens I3 filtrates

❖ *Preparation of filtrates and pathogen suspensions*

B. amyloliquefaciens I3 filtrates were obtained from the cultures growing in PDB media

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(Potato Dextrose Broth). Sixteen pre-cultivated pastilles of *B. amyloliquefaciens* I3 in PDA media (Potato Dextrose Agar) were placed in 200 ml of PDB for 48 hours. Cultures of *B. amyloliquefaciens* were also prepared in confrontation with G1-1 and A5-1 strains of *Z. tritici*. The cultures were incubated in the dark under agitation at 25°C for five days and then sterilized by filtration (0.45 μm (Minisart filters/Sigma-Aldrich)).

The suspensions of *Z. tritici* strains (G1-1 and A5-1) were obtained after seven days of incubation in the dark at 18°C on PDA media. The suspension concentration was adjusted to 10^6 pycnidiospores/ml.

❖ *Antibiotic activity quantification*

The three filtrates (F1 prepared with I3, F2 prepared with I3 and G1-1, and F3 prepared with I3 and A5-1) and the control F4 (the filtrate was substituted by sterile distilled water) were tested in three dilutions (d1=1/10, d2=1/2, and d3=9/10) on both *Z. tritici* strains G1-1 and A5-1. Depending on the dilution, the filtrate was mixed with 100 μl aqueous suspension of *Z. tritici* supplemented with PDB media for a final volume of 1 ml. The final suspension was deposited a 96-well plate with 300 μl per well. For each modality, three biological repetitions with three technical repetitions were performed. The control F4 was prepared with sterile MilliQ water.

The antibiosis effect was characterized by nephelometry (Chronos-NEPHELO star plus, BMG LABTECH) at 25°C. The number of analysis cycles was 16, with 6 hours between two successive cycles and 80% of laser intensity. Before each measurement, the plate was shaken for 300 seconds at 150 rpm.

❖ *Statistical analysis*

SPSS 21 statistic software was applied for turbidity data analysis. Statistical analyses were established at three factors: the first corresponded to the filtrates (F1, F2, F3, and the control F4), the second factor indicated the dilutions (d1, d2, and d3) and the third factor denoted the *Z. tritici* strains (A5-1 and G1-1). ANOVA was applied for the analysis of variation of means, while the Duncan test was used for the comparison of means at $p = 0.05$.

c) *Production and identification of secondary metabolites*

❖ *Culture preparation*

In petri plates containing the 24-hour-old *Z. tritici* culture, 10 μl of the pre-cultivated *B. amyloliquefaciens* culture on PDA media for 48 hours was added to the centre of the plates. These latter were incubated at 25°C in the dark for four days.

The liquid cultures were prepared according to the same protocol described in the previous section.

❖ *Secondary metabolites extraction*

The secondary metabolites were extracted from the agar media by mixing three agar fragments randomly collected from the inhibition areas or the I3 culture with 2 ml of methanol in assay tubes. The mixture was homogenized using vortex and incubated at 4°C for 4 hours, then centrifuged for 10 min at 4000 rpm. The supernatant was recovered and purified by filtration (Millipore of 0.45 μm).

The liquid-liquid extraction was carried out by precipitation of the secondary metabolites at pH=2 (adjusted with hydrochloric acid (HCl)) and then centrifugation (20 min at 8000 rpm) followed by two successive rinses of the precipitate (ultra-pure water at pH=2), methanol extraction, and filtration (Millipore of 0.2 μm). The extract was dried by rotavapor and re-suspended in 1 ml of 0.01 M PBS.

Regarding the PBS buffer, 8 g of sodium chloride (NaCl), 0.2 g of potassium chloride (Kcl), 1.4 g of dibasic sodium phosphate (Na_2HPO_4), and 0.24 g of di-potassium phosphate (K_2HPO_4) were added to one litter of ultra-pure water. Then, the pH was adjusted at 7.4.

❖ *Lipopeptides and polyketides identification by HPLC/MS*

The extracts were analyzed by HPLC-MS (Thermo Scientific) using a C18 column. After, a 10 μl injection of each extract (diluted at 1/100), the elution was conducted in a binary solvent system (solvent A: water + 0.1% formic acid and solvent B: acetonitrile + 0.1% formic acid) with the following gradient: 30% solvent B for 5 min, from 30% to 45% solvent B for 5 min and from 45% to 100% solvent B for 25 min, (flow rate was 0.5 ml/min at 40 °C). The detected lipopeptides and polyketides were identified according to their molecular weight.

III. Results

a) *Inhibition of pycnidiospores germination by Bacillus amyloliquefaciens filtrates*

All the tested filtrates showed an antifungal effect against the pycnidiospores of *Z. tritici* G1-1. These results were confirmed by the nephelometry turbidity measurements, as demonstrated in figures 1, 2, and 3. The pycnidiospore's germination rate was reduced progressively with the decrease of the filtrates dilution factor. Also, the ANOVA revealed a highly significant difference at $p=0.05$ between filtrates and dilutions. The average comparison by Duncan test classified the filtrates (F1, F2, and F3) and the control (F4) into four homogeneous groups. Filtrates F1, F2, and F3 showed very high levels of inhibition compared to the control (F4). However, it should be noted that F1, prepared with only *B. amyloliquefaciens* I3 caused a high inhibition of pycnidiospores germination compared to F2 (prepared with I3 and *Z. tritici* G1-1) and F3 (prepared with I3 and

Z. tritici A5-1) filtrates on the three tested dilutions (1/10, 1/2 and 9/10). After 96 hours of incubation, the dilutions d2, and d3 of both F1 and F2 filtrates inhibited 95% (101.2 KNTU) of pycnidiospores. The dilution d1 of F1 inhibited 82% (358.6 KNTU) of pycnidiospores germination compared to the control, while the same dilution of F2 did not exceed 42% of inhibition (1148.7 KNTU) (Figures 1 and 2). The dilutions d1, d2, and d3 of F3 expressed inhibition levels of 53% (942.5 KNTU), 64% (711.1 KNTU), and 99% (190 KNTU) respectively (Figure 3).

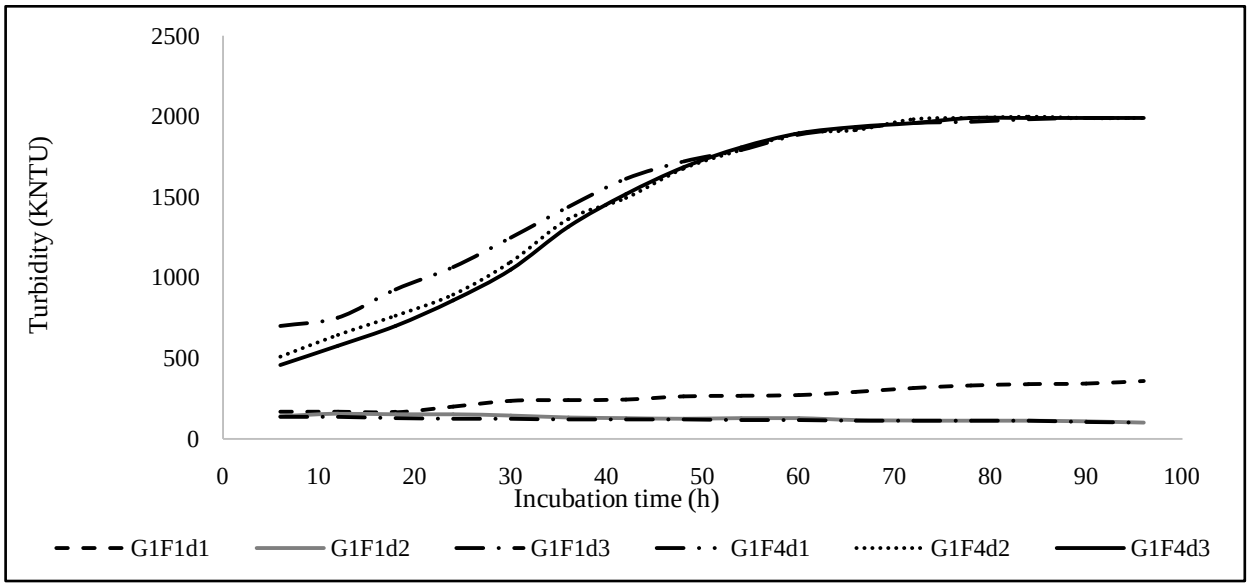

Figure 1: Kinetic of *Zymoseptoria tritici* G1-1 pycnidiospores germination in the presence of the F1 filtrate (prepared with *Bacillus amyloliquefaciens* I3) at the three dilutions (1/10, 1/2 and 9/10) in comparison with the control (F4). NTU: Nephelometric Turbidity Unit.

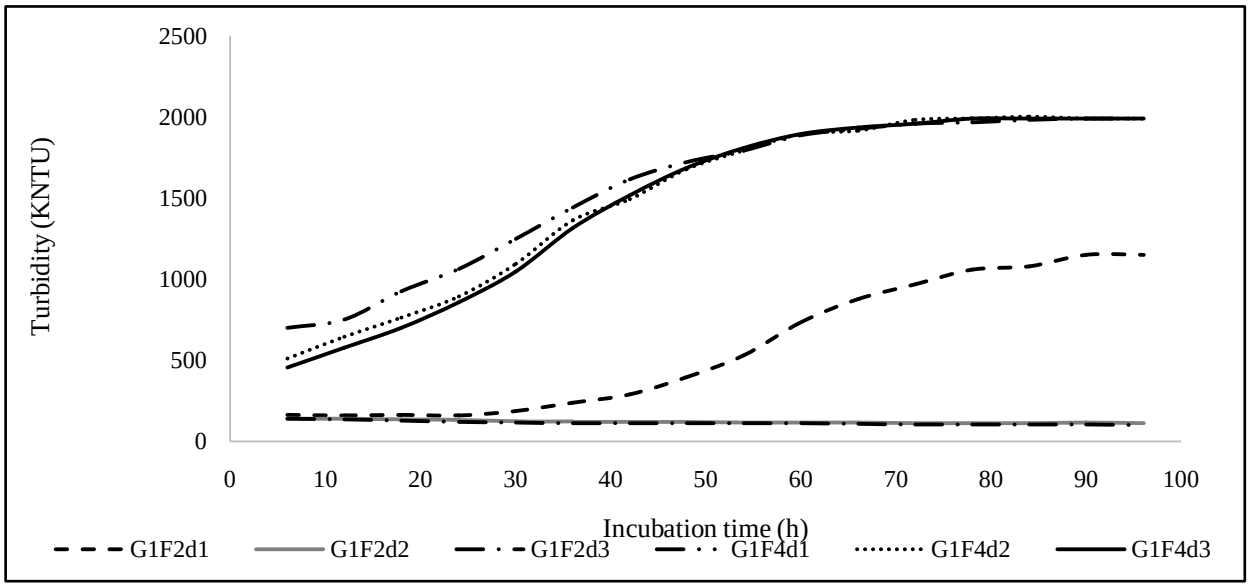

Figure 2: Kinetic of *Zymoseptoria tritici* G1-1 pycnidiospores germination in the presence of the F2 filtrate (prepared with *Bacillus amyloliquefaciens* I3 and *Zymoseptoria tritici* G1-1) at the three dilutions (1/10, 1/2 and 9/10) in comparison with the control (F4).

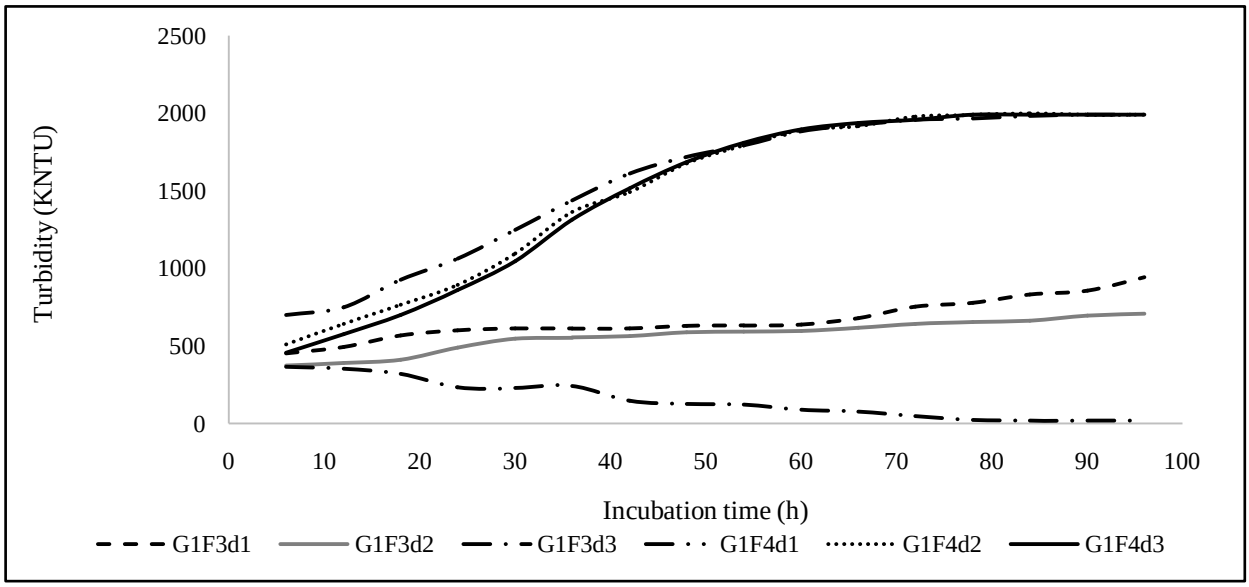

Figure 3: Kinetic of *Zymoseptoria tritici* G1-1 pycnidiospores germination in the presence of the F3 filtrate (prepared with *Bacillus amyloliquefaciens* I3 and *Zymoseptoria tritici* A5-1) at the three dilutions (1/10, 1/2 and 9/10) in comparison with the control (F4).

Regarding the *Z. tritici* A5-1, the antagonistic effect of the I3 strain on the germination rate of *Z. tritici* A5-1 was then studied by nephelometry using the I3 filtrates F1, F2, and F3 (figures 4, 5, and 6). ANOVA analysis of turbidity data showed that the inhibitory effect of *B. amyloliquefaciens* I3 filtrates, without or with *Z. tritici* G1-1 and A5-1 strains, depended on the tested dilutions. After 96 hours, dilution d1=1/10 caused an inhibition rate of pycnidiospores germination around 88% (244583 NTU), 33% (1335620 NTU), and 51% (991081 NTU) for F1, F2, and F3 filtrates, respectively. For the medium dilution (d2=1/2), the inhibition percentages were about 93% (132210 NTU), 90% (201164 NTU), and 57% (796998 NTU) for F1, F2, and F3 filtrates, respectively. The dilution d3=9/10 exhibited the highest inhibition rates reaching the 94% (108850 NTU) for F1 and F2, and 72% (566249 NTU) in the case of F3 (Figures 4, 5 and 6).

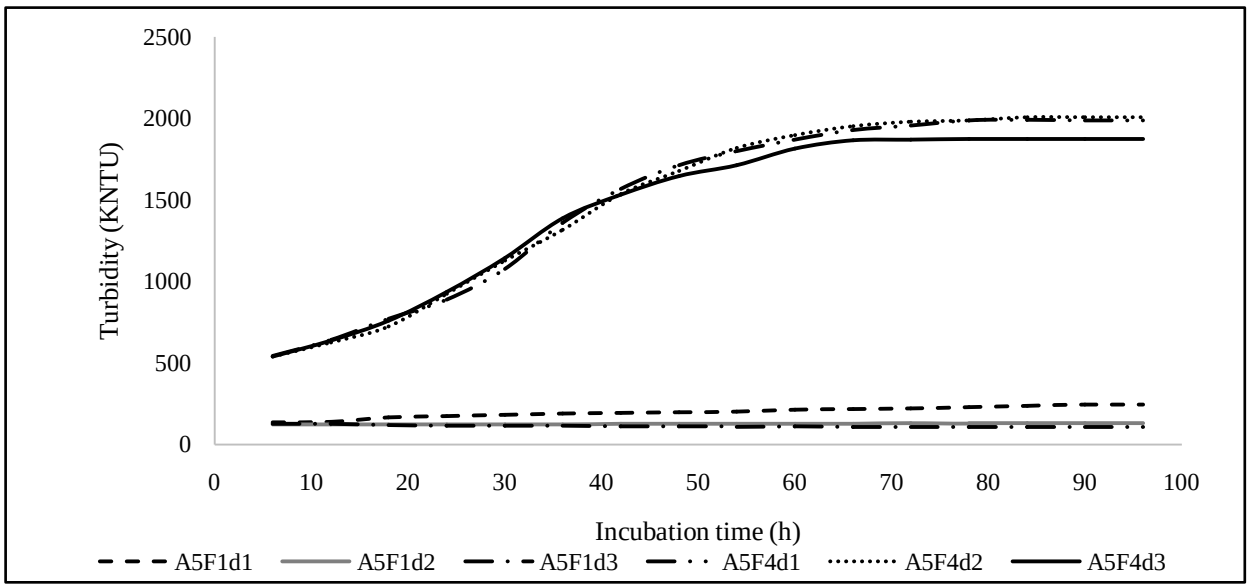

Figure 4: Kinetic of *Zymoseptoria tritici* A5-1 pycnidiospores germination in the presence of the F1 filtrate (prepared with *Bacillus amyloliquefaciens* I3) at the three dilutions (1/10, 1/2 and 9/10) in comparison with the control (F4).

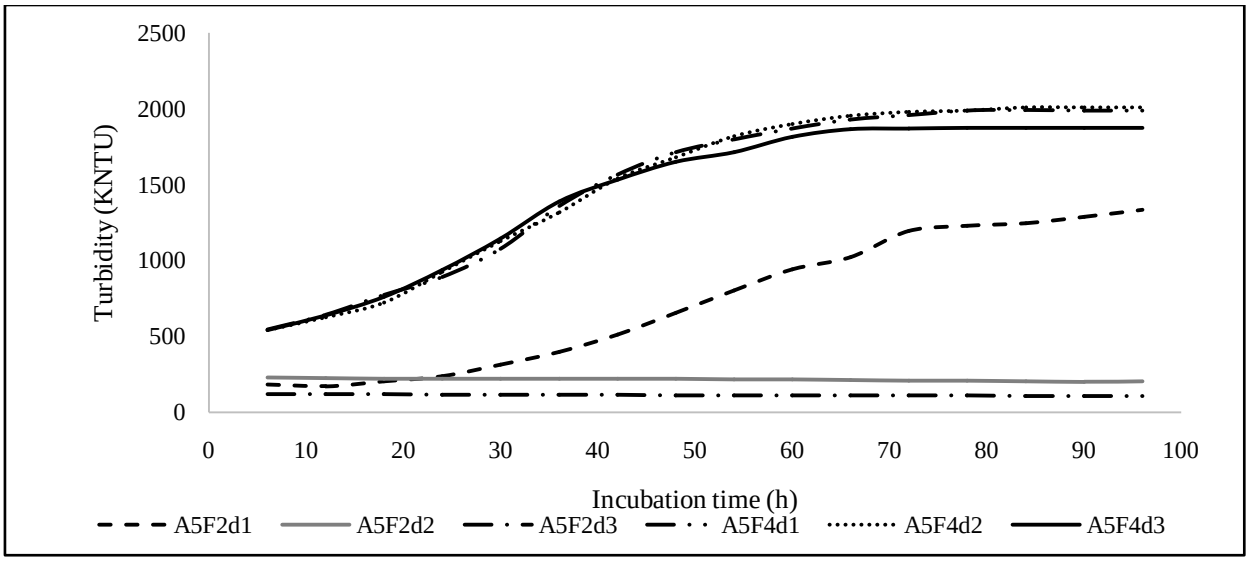

Figure 5: Kinetic of *Zymoseptoria tritici* A5-1 pycnidiospores germination in the presence of the F2 filtrate (prepared with *Bacillus amyloliquefaciens* I3 and *Zymoseptoria tritici* G1-1) at the three dilutions (1/10, 1/2 and 9/10) in comparison with the control (F4).

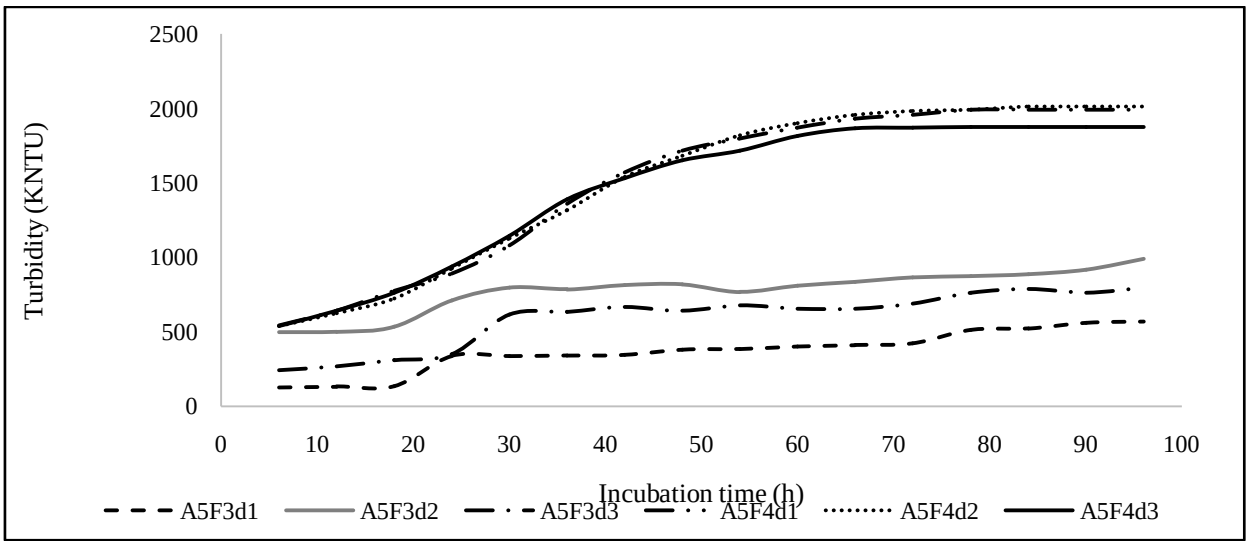

Figure 6: Kinetic of *Zymoseptoria tritici* A5-1 pycnidiospores germination in the presence of the F3 filtrate (prepared with *Bacillus amyloliquefaciens* I3 and *Zymoseptoria tritici* A5-1) at the three dilutions (1/10, 1/2 and 9/10) in comparison with the control (F4).

b) Antifungal compound identification

The HPLC-MS characterization of the metabolic composition of both agar culture and liquid filtrate extracts has revealed the presence of several families of cyclic lipopeptides and polyketides (see table 1). Comparing the molecular weights to already known lipopeptides [17,18,19], three major families of cyclic lipopeptides (surfactins, fengycins, and iturins) and three families of polyketides (macrolactins, chlorotetaines, and bacillaenes) were identified in the extracts prepared from inhibition zones of *Z. tritici* in agar media and liquid filtrates of *B. amyloliquefaciens* I3 without or with *Z. tritici* G1-1 and A5-1 strains. The identified lipopeptides include surfactins C12 and C15 with molecular weights of 1015.4 Da (Dalton) and 1057.5 Da, respectively. Two variants of iturins were also produced by *B. amyloliquefaciens* I3, named iturins A or mycosubtilin with a chain of 14 and 15 carbon atoms (C14, C15) whose molecular weights were 1043.6 Da and 1057.6 Da respectively, and iturins B C14 and C15 with a molecular weight of 1065.6 Da and 1079.3 Da, respectively. The macrolactins produced by the strain I3 are the following: D, A28, 7-o-succinyl-A, and 7-o-malonyl-A, with molecular weights ranging from 510.5 Da to 628.6 Da. The two identified chlorotetaine isoforms Cl35 and Cl37 had molecular weights of 289.2 Da and 291.1 Da, respectively. Also, two bacillaenes were identified, bacillaene A (582.5 Da) and bacillaene B (582.4 Da). Regarding the fengycin family, the only homologous produced by strain I3 was fengycin A with

17 carbon atoms and a molecular weight of 1498.8 Da. The families of iturins, macrolactins, bacillaenes, and chlorotetaines were the most abundant compared to the other families. However, extraction from the inhibition zones on agar media allowed higher intensities of the molecules produced by I3 strain - in the presence and the absence of *Z. tritici* G1-1 and A5-1 strains - compared to liquid culture extraction. The mass-spectrum and the identified families of lipopeptides and polyketides are presented in Table 1.

Table 1: Antifungal metabolite production of *Bacillus amyloliquefaciens* I3 detected by HPLC-MS.

Metabolite families	Identified molecules	Molar mass (Da)	Peak area (x10^5)					
			Agar media			Liquid media		
			I3	I3+G1	I3+A5	I3	I3+G1	I3+A5
Iturins	A C14	1043.6	9.05	3.32	2.70	7.94	1.17	0.39
	A C15	1057.6	5.52	1.09	1.04	3.21	0.45	0.568
	B C14	1065.6	5.42	4.32	1.41	2.29	0.534	3.12
	B C15	1079.3	77.7	6.27	2.61	8.01	21.5	2.64
Bacillaenes	A	582.5	2.89	2.67	1.37	1.89	1.80	2.94
	B	582.4	10.6	1.77	5.57	6.78	2.67	2.15
Chlorotetaines	Cl35	289.2	7.95	1.77	1.40	2.10	0.95	0.47
	Cl37	291.1	8.20	1.67	8.66	3.30	0.99	0.61
Macrolactins	D	628.6	74.5	4.06	1.07	7.05	2.82	0.23
	A28	425.4	1.61	16.7	-	2.40	3.39	-
	7-o-malonyl-A	524.6	21.9	2.44	0.90	8.45	3.66	0.31
	7-o-succinyl-A	510.5	2.80	9.78	-	4.74	0.57	0.52
Fengycin	A C17	1498.8	0.30	0.12	-	0.21	0.11	-
Surfactins	C12	1015.4	0.29	0.21	0.22	0.19	0.05	-
	C15	1057.5	0.20	0.19	-	0.11	0.10	0.28

IV. Discussion

The results of the inhibition assays of pycnidiospores of *Z. tritici* strains by *B. amyloliquefaciens* I3 (pycnidiospores of both *Z. tritici* strains, obtained from soft and durum wheat) have shown the presence of antifungal metabolites that are involved in the antagonistic activity of *B. amyloliquefaciens* I3 against *Z. tritici* (antibiosis).The three tested filtrates (F1, F2, and F3) induced inhibition of *Z. tritici* pycnidiospores germination up to 99%. However, dilutions d2 and d3 (1/2 and 9/10) showed inhibition rates ranging from 94% to 99% compared to the negative control F4. The identified property of *B. amyloliquefaciens* I3 to inhibit *Z. tritici* illustrate a new significant antifungal activity of strain I3. Our finding is consisted with reports by Zhang et al., [20] and Dimkic et al., [21] describing a high antagonistic effect of the crude extract of *B. amyloliquefaciens* TF28 lipopeptides against *F. oxysporum*, *B. cinerea, and Pythium sp*. Similar results were obtained by Sun et al., [19] who demonstrated that the *B. amyloliquefaciens* ES-2 filtrate inhibited significantly the growth of several phytopathogenic fungi such as the following: *Penicillium italicum*, *Fusarium culmorum*, *Botrytis cinerea*, *Magnaporthe grisea*, and *Erysiphe graminis hordei*. Considering the same context, Xu et al., [22] confirmed the severe toxicity of the *B. amyloliquefaciens* SQR9 filtrate on *F. oxysporum* conidia. Likewise, the filtrate of *B. amyloliquefaciens* CNU114001 inhibited the germ tube elongation of *B. cinerea*. The same bacteria showed a broad-spectrum of antagonistic activity against 12 phytopathogenic fungi (*Alternaria panax*, *B. cinerea*, *Colletotrichum acutatum*, *C. orbiculare*, *Corynespora cassicola*, *F. oxysporum*, *Phytophthora capsici*, *P. digitatum*, *Rhyzoctonia solani*, *Stemphylium lycopersici*, *Pyricularia grisea*, and *Sclerotinia sclerotiorum*) [23].

To characterize the responsible compounds involved in this inhibition, several identification methods were used, and the most important was based on chromatographic techniques. The HPLC-MS results of the different types of *B. amyloliquefaciens* I3 extracts, in both the presence and absence of *Z. tritici* A5-1 and G1-1, showed the production of many molecules with very high antimicrobial activity, including iturins, macrolactins, bacillaenes, chlorotetaines, fengycins, and surfactins. However, iturins A and B were identified in all extracts from I3 alone or in confrontation to *Z. tritici* G1-1/A5-1 strains with a very high relative abundance. Similar results were obtained in several previous investigations, among them the study of Arrebola et al., [24] which showed that iturin A produced by *B. amyloliquefaciens* PPCB004 affected *A. citri*, *Botryosphaeria sp*., *C. gloeosporioides*, *Fusicoccum aromaticum*, *Lasiodiplodia theobromae*, *P. crustosum*, and *Phomopsis persea* while the other lipopeptides - fengycins and surfactins -produced by this antagonist did not have a major effect on all studied pathogens. Inhibition of these seven fungal species could be added to nine other fungal pathogens affected by iturin A produced by different strains of Bacillus, as described by Hsieh et al., [25]. According to Jacques, [26], the fungitoxic activity of iturins is due to their ability to

penetrate membranes. Pathak, [27] proved that fengycins have a high fungitoxic activity, specifically against filamentous fungi. They also contribute to the formation of a complex with sterols, which suggests the ability of fengycines to interact with membrane lipids. The antifungal activity of fengycins is enhanced by the presence of surfactins [28,19], and iturins [29]. In this regard, Raaijmakers et al., [30] reported that surfactins contribute to the formation of a stable biofilm on host surfaces, protecting bacteria against antibiosis and competition of other microorganisms. While surfactins are not very active directly on fungal pathogens. The secretion of these lipopeptides promotes the colonization of root tissues by bacteria, which is a necessary condition for the constant availability of antifungals and the successful biocontrol of plant pathogens [5,31]. Furthermore, Xu et al., [22] demonstrated that bacillomycin D (a type of iturin) produced by *B. amyloliquefaciens* SQR9 contributes to biofilm formation in addition to its antifungal activity against *F. oxysporum in vitro* and *in vivo*. This may explain the low production of fengycins and surfactins molecules in the confrontation between *B. amyloliquefaciens* I3 and *Z. tritici* G1-1 and A5-1. In addition to lipopeptides, polyketides (macrolactins, bacillaenes, and chlorotetaines) were identified with high intensities in the different studied extracts. Thus, the compounds are also responsible for the inhibition of *Z. tritici* due to their high intensities when confronted with *Z. tritici* strains. Furthermore, the results obtained from the two studied extraction techniques have shown no difference in the metabolites produced in both the presence and absence of *Z. tritici*. However, in extracts prepared from I3 alone, the relative abundance of iturins, chlorotetaines, bacillaenes, and macrolactins was more significant compared to the other extracts prepared from I3 confronted to G1-1 and A5-1.

V. Conclusion

The *B. amyloliquefaciens* I3 filtrates tested in this study demonstrated high antifungal activity against *Z. tritici*. This effect was correlated to the importance and the diversity of the identified antifungal metabolites in the filtrates. These interesting results obtained in this study justify the need to proceed for purification of these metabolites and to test them against Septoria and other diseases of wheat *in planta*. If these metabolites are effective in plants, it would be useful for conducting advanced research to develop biopesticides from these molecules and testing them in other pathosystems.

Acknowledgments

We thank the Ministry of National Education, Vocational Training, Higher Education, and Scientific Research of the Kingdom of Morocco for funding the Arimnet research project entitled “Bacplant,” and we are also thankful for the National Center for Scientific and Technical Research (Morocco) for its financial support within the framework of the excellence research grant program. We thank the financial support from the French ANR (Agence National de la Recherche) project LabCom, ESTIM, and the ERASMUS funding for the Agrocampus-Ouest and Hassan II Institute of Agronomic and Veterinary medicine, French–Moroccan collaboration.

Conflicts of interest

The authors declare no conflicts of interest.

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Global Journal of Science Frontier Research: D
Agriculture and Veterinary
Volume 20 Issue 1 Version 1.0 Year 2020
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Awareness of Mango Farmers at Southern Ethiopia on the Pest Status and Current Management Practices for the Control of the Fruit Flies (Diptera: Tephritidae)

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Abstract- Fruit flies generally considered as the most devastating pest of fruits and vegetables. The invasive fruit fly, Bacterocera dorsalis, expected to be introduced to Ethiopia in 2005. The awareness of mango farmers from Gamo and Wolaita districts in Ethiopia on the pest status and the current management options adopted for the control of this pest was followed by the use of a questionnaire. The survey results indicated that Ethiopian farmers rank fruit flies among the major pests of mango in Ethiopia. Farmers generally believed that it is more damaging than other insect pests of mango. Possible losses such as loss of market value and rejection of produce at the local market were also reported by the farmers. Several tactics are being adopted by farmers for the control of fruit flies in Ethiopia. These tactics include the use of insecticide, cultural control measure and, the use of trappings to manage fruit flies. Some of the respondents use a combination of insecticides and cultural practices to reduce the menace of fruit flies. It was evident that farmers adopt multiple tactics to minimize the losses due to fruit flies in an IPM fashion.

Keywords: *survey, fruit flies, management, gamo, wolaita.*

GJSFR-D Classification: *FOR Code: 070106*

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I. Introduction

Fruit flies (Diptera: Tephritidae) are among the most important pests of fruit and vegetables worldwide. They constitute one of major threats to horticultural production, causing substantial produce losses in East, Central, and West Africa (White and Elson-Harris, 1992; Muhammad and Kiilu, 2004; ICIPE, 2007; Van Mellett *et al*., 2007). The family includes more than 5000 species worldwide, approximately 1400 species of which develop in fleshy fruits (Norrbom *et al.,* 1999). Sub-Saharan Africa is home to 915 fruit fly species from 148 genera, with 299 species evolving in either wild or cultivated hosts or in both (Ekesi, 2010). The common fruit fly species in Ethiopia are *Ceratitis fasciventries*, *Ceratitis cosyra,* and *Bactrocera invadens* (Dawit *et al.*, 2015). They cause enormous economic losses in every part of the world where fruits and vegetables are grown. Economically important tephritid fruit flies worldwide can be found in five genera: *Anestrepha, Bactrocera, Ceratiris, Rhagoletis, and Dacus (*White and Elson-Harris, 1992). The fruit fly *Ceratitis cosyra* has been long recognized as the most damaging tephritid fruit fly pest of mango (*Mangiferaindica*) in Africa, including Ghana (Lux *et al.,* 2003). However, in 2003, a new species *Bactrocera invadens* Drew *et al.* invaded Africa from the Indian subcontinent (Mwatawala *et al.,* 2004, Drew *et al.,* 2005). Within a span of few years, the species rapidly spread across Africa and was detected in Ghana in 2005 (Billah*et al.,* 2006). Mango is considered the primary host of *B. invadens* (Ekesi and Billah 2003*,* Mwatawala 2009). Yield loss of 15- 50% in mango was reported from some African countries, especially in West Africa (Vayssierres*et al.,* 2006).

Mango (*Mangiferaindica* L.) is the most widely cultivated fruit tree in the Sahel and one of the most important tree crops in the tropics (Deng and Janssen, 2004). It is a highly prized exotic fruit on the European market and one of the important fruit crops grown in tropical and sub-tropical regions (Nakasone and Paull, 1998; Nofal and Haggag, 2006). World production of mango in 2005 was estimated at 28.51 million tonnes (Mt) (Evans, 2008). Of this, Africa produced only 2.5 million tonnes, accounting for about 10 percent of fresh fruits and 11 percent of processed mango. The area coverage under mango in eastern Ethiopia has reached about 35% of the total acreage allotted for fruit production (Yeshitla, 2004). According to FAOSTAT (2010), the total cultivated area for mango in Ethiopia is not more than 12000 hectares. The highest annual production estimate in the past five years is 180,000 Mt, and more area coverage is expected in the south-western and other parts of the country due to more conducive climatic and edaphic factors.

The awareness of Ethiopian mango farmer son the pest status, and current management options for the control of this pest was studied in two districts in the Gamo and Wolaita district of Southern Ethiopia. The Objectives of this study were to assess the general awareness of mango farmers on the pest status of fruit flies and to study the management practices adopted by the farmers for the management of the pest.

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II. Materials and Methods

a) Field surveys

A field survey was conducted between October 2018 and January 2019 to establish the perception of mango farmers on the pest status and current management options for the control of *fruit flies* in Southern Ethiopia. Semi-structured questionnaires were administered to farmers selected at random, with the majority being members of the banana and mango producers. The study was conducted in two districts of Southern Ethiopia, namely the Gamo and the Wolaita, where fruit flies were previously reported as being prevalent by the Arbaminch plant protection laboratory, Ethiopia. In each district, a local Kebele was selected; Chano Mille and Chano Chalba in the Gamo and Bolloso Sore and Kindo Koyisha from Wolaita district. One hundred four (104) farmers were selected for the study, with each selected farmer having a farm size of at least 2 ha. The stratified random sampling procedure was adopted for the study so that each mango producing village in the selected local Kebele represented a stratum (sampling unit). Farmers were selected at random from each of the sampling units. Criteria for selection include the farmer being in production for at least four years. Where applicable, farmer registration to local Kebele was sought to confirm their status. This is because the level of awareness of members of the group is high due to their export disposition, which ensures the adoption of reasonably fair technologies that will guarantee the production of high-quality fruits. Local Kebele officials, therefore, assisted in the selection of most of the sampling units. Questions in the questionnaire were premised on finding information on pest problems commonly encountered by farmers in mango fruit production as well as finding the major and minor pests. Questions were also asked relating to the awareness of fruit flies, their species composition, and the nature of the damage caused by fruit flies.

Farmers were also asked to rate the effect of fruit fly on fruit production relative to other arthropod pests commonly encountered in the mango agro-ecosystem. Question relating to knowledge of the economic significance of the species with regards to it being a quarantine pest, and the losses it could cause in the mango industry were asked in the questionnaires. They were requested to indicate whether fruit flies were an exotic, endemic, and/ or occasional pest. The concluding aspect of the questionnaire dealt with matters relating to management options adopted by farmers.

b) Data analysis

All data generated from the field survey (questionnaire) were analyzed using descriptive statistics (percentages).

III. Results

a) Awareness of mango farmers on the pest status of fruit flies

Results from the survey questionnaire indicated that all the respondents (100%) have encountered some sort of pest problem at a point in their career as mango producers. Several insect pests were listed by the respondents as being pests in mango in Ethiopia. The insect pests mentioned by the farmers grouped under two set, namely major and minor pests (Table 1). Farmers categorized pests as being major mostly based on the length of time they spend dealing with them on their farms over the production period and the extent of intervention required in terms of monetary values. Few 8 (7.69%) of the farmers were thought that scale insects and thrips were of major concern in the mango plantation. The majority of the respondents 80 (76.9%) indicated that fruit flies were of major economic importance causing damage that can lead to the production of unmarketable fruits. The second in order of significance as a major pest to 78 respondents (75.0%) were the mealy bugs. This proportion of farmers believed that, mealy bags caused a lot of problems leading to yield reduction in the mango enterprise. They were fully aware of fruit flies being pest of economic significance. However, some farmers simply dismissed fruit flies as houseflies that are just opportunistic and taking advantage of the abundant food (rotting) found at the peak period of harvest. To this group, no harm was done to the fruit as the result of their presence. Similarly, 76 of the respondents (73%) also indicated mango stone weevils as being major pests that caused a significant reduction in fruit quality.

Table 1: Insect pests faced by farmers on mango farms

Name of insect (n=104)	Percentage (%)
Major	
Fruit flies	76.90
Mealy bugs	75.00
Stone weevil	36.50
Scale insects	7.69
Termites	2.80
Minor	
Fruit flies	3.80
Grasshopper	8.80
Mites	4.70
Ants	22.8

Few farmers cited termites (2.8%) and ants (27.2%) as pests of major economic importance that required some attention during and even after the production cycle. Insect pests indicated as minor pests by the respondents during the survey included fruit flies (3.8%), grasshoppers (8.8%), mites (4.7%) and ants (22.8%).

b) Fruit fly species are known to the farmers

Four fruit flies species were known to the farmers in the study area. These species were *Ceratitis cosyra, C. fasciventries, Bacterocera cucurbitae* and *B. invadens.* Forty-eight percent (68.1%) of the farmers indicated that they knew some species of fruit flies: of these, 39.2 % attested to knowing *C. cosyra* and 18.8% to *B. invadens*. This is an indication that a reasonable number of the farmers are already aware of the presence of the African invader fly relative to other species in spite of its recent introduction and establishment in Ethiopia.

In addition to insect pests, farmers mentioned some diseases which affect the productivity and quality of their mango product. The measure diseases they mentioned include anthracnose die back, and root rot are the measure one. They mentioned that anthracnose affects the leaf, flower, and the immature fruits, and the mature fruit loses quality.

c) Types of damage caused by fruit flies

The perceptions of farmers on the types of damage caused by fruit flies also vary significantly (Fig. 1). About 45.0% of them mentioned fruit destruction as one of the damage caused by the fruit flies. Some 27.4% of the farmers believed that, the fruit flies pierced the skin of the fruit and lay the egg and the egg changed in to larvae. Others (11.2%), indicated that the species caused fruit rottening, while 5.5% of the farmers indicated that a change in colour resulted from the attack by the fruit fly, and this led to premature ripening of the fruit. Total loss in yield is the direct effect of the presence of mango fruit fly in the mango production to some respondents (2.6 %), because the flies caused total destruction of the fruit leading to complete loss of yield in the absence of some intervention measures to control them.

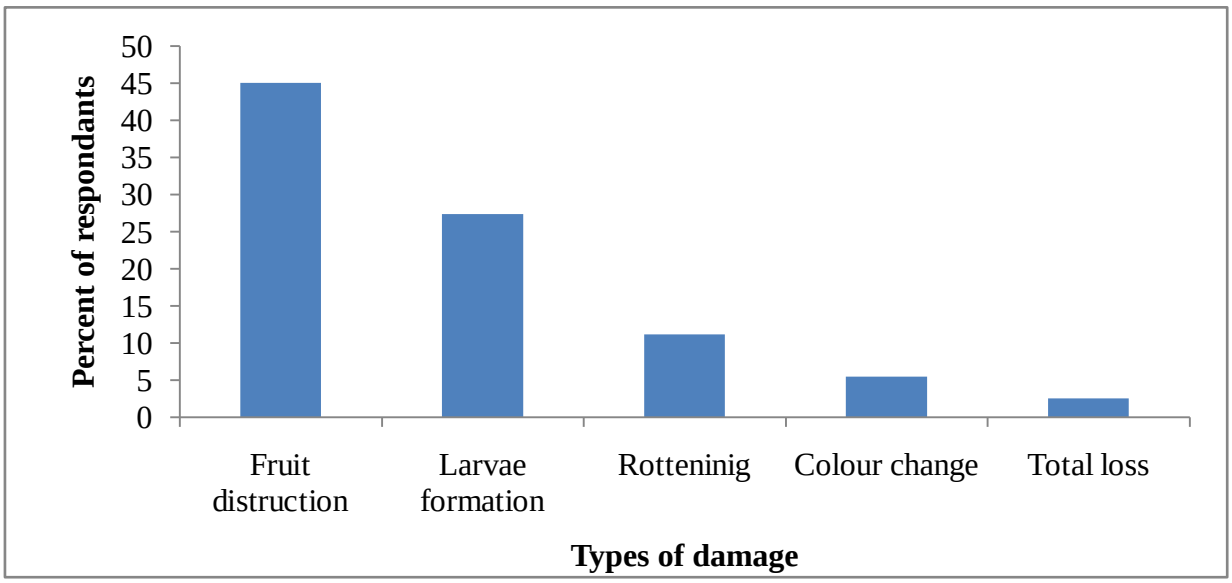

Figure 1: Percentage of respondents on types of damage on mango fruit due to fruit flies (n= 104).

d) Effects of fruit flies on fruit production

Generally, majority of the farmers (67.5%) are of the opinion that, the fruit flies caused very severe damage to the mango production. This implies that mango producers in Ethiopia are aware that, the fruit flies can cause serious damage to their crops with detrimental consequences to their incomes. On the rating of the mango fruit flies relative to other pests in the mango plantation, 70.0 % of the respondents indicated that the flies were more damaging to their fruit. Thus, mango farmers are aware of the threat posed by the fruit flies to the mango production.

e) Losses caused by the fruit flies

A greater number of the respondents (76.2%) revealed that, the presence of the fruit flies in Ethiopia causes some massive losses to farmers. These losses (fig. 2) ranged from a loss of market value (64.1%), loss in quality of the fruits (71.3%), rejection of fruits at local markets (49.1%), and increase in the cost of production (7.7%).

f) Pest status of fruit flies

Responding to the question on the pest status of the fruit flies, 28.4% of the respondents believed that the pest was a common one i.e., it has been in the system since they started the mango fruit production. On the other hand, 58.5% of the farmers said it was an unusual pest that found itself unto the country's landscape some ten years ago. Similarly, some 14.6% of the respondents firmly believed that it was an occasional pest occurring only when there was excess fruit in the area. About 36.9% of the farmers indicated that the pest was associated with newly introduced improved mango varieties.

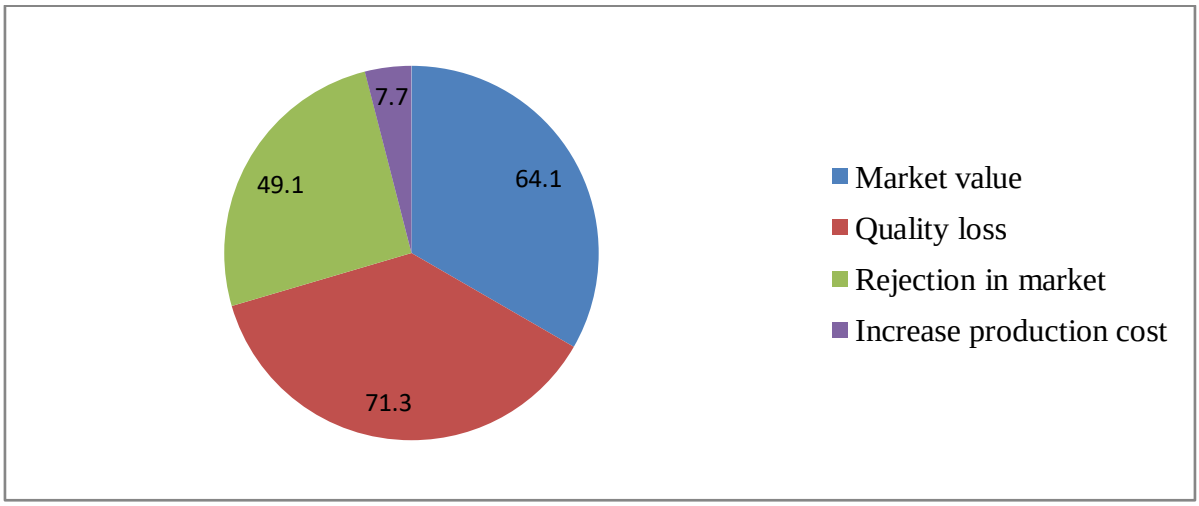

Figure 2: Response on nature of losses experienced by mango farmers (n=104)

g) Management strategies implementing by Ethiopian farmers to control fruit flies

Most of the respondents have used one control measure or the other to reduce the effects of fruit flies in their effort to produce fruit that will meet the needs of their customers. Two control methods, namely chemical and cultural, were dominant among all the respondents (fig. 3). Some 11.5% of the farmers apply chemicals as either a single control method or together with one or more other control measure(s). Similarly, (76.9%) of the farmers adopted cultural control measures e. g. fallen fruit destruction, branch pruning, and farm sanitation to control the fly. All the respondents were oblivious of any careful use of resistant varieties for the management of the fruit flies in Ethiopia. They generally believed that no variety of mango was in any way resistant to the attack of the pest and hence the use of host-plant resistance as means of controlling would be ineffective for all practical commercial purposes. Some 28.5% (from Gamo district) of the farmers showed to the use of trapping for the reduction of the male fruit flies numbers. Many NGO's and Arbaminch plant health clinics work on the management of fruit flies in the Gamo area, and they providing a lure trap to minimize the male population. A significant number (56.9%) used insecticides, and traps, in combination on their farms to combat the menace of fruit flies. Similarly (46.5%) used insecticides alongside cultural practices like collection and burial of fallen fruits to maintain better sanitary conditions on their farms. This in essence, has the advantage of reducing the source of the infestation. Some farmers (72.3%) used a combination of insecticides, traps and cultural methods for the fruit flies control (fig. 3).

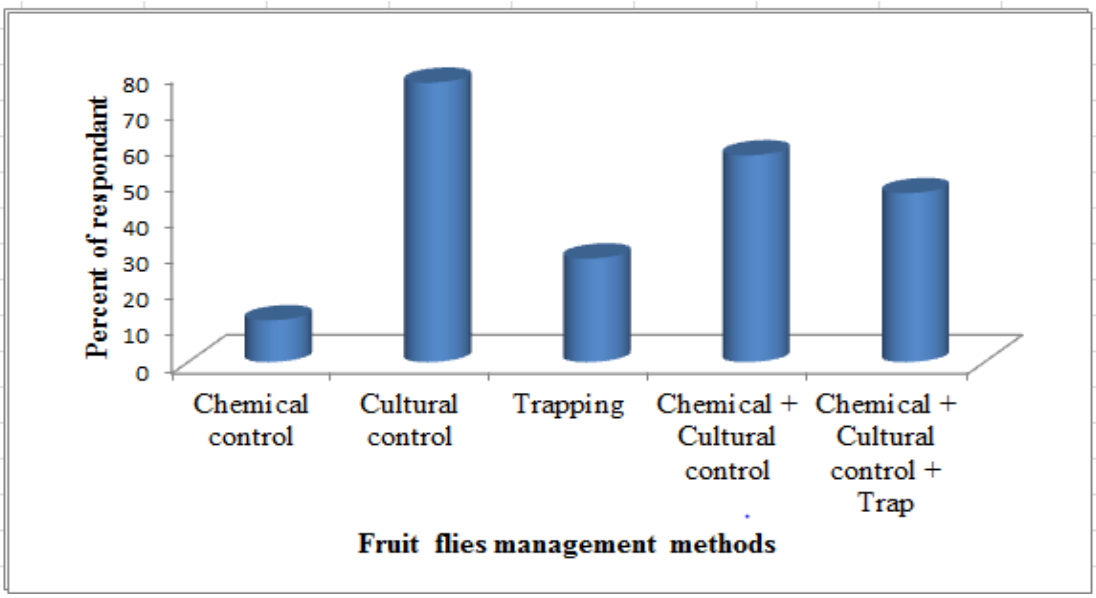

Figure 3: Percentage adoption of different management methods by farmers to control fruit flies in the study area (n=104).

IV. Discussion

Farmer Perceptions of pest status and Management option for fruit flies control

The results of the survey indicated that mango farmers rank fruit flies among the major pests of mango in Sothern Ethiopia (Table 1). This is an indication that Ethiopian mango farmers are already aware of the potential damage of the mango-infesting fruit flies. This confirms Vayssieres *et al*., (2005) observation that losses caused by fruit flies range from 12- 50% for mangos in Benin, depending on the season and management practices adopted. Thus, fruit flies inflict heavy losses on fruits and vegetable crops because of their phytophagous habits (Norrbom *et al*., 1999). Activities by different fruit fly species lead to these loses and vary between fruit fly species, fruit hosts involved, and between communities. Thus, they are accorded different economic statuses in different farming systems in the world (Mwatawala *et al*., 2009). This knowledge could have been gained as a result of curiosity on the part of the farmers trying to know the identity of flies they see most often or through contact with extension workers and some NGOs in their area. Several strategies are being adopted by farmers for the control of fruit flies in Ethiopia. These strategies include the use of insecticides (11.5%), cultural control measures (76.9%), and use of trappings (28.5%) as strategies to manage fruit flies. While46.5% of the respondents use a combination of insecticide, and cultural practices to reduce the threat of fruit flies. It was evident that farmers adopt multiple strategies to minimize the losses due to fruit flies in an IPM fashion outlined by Ekesi and Billah (2006) and Obeng-Ofori (2007). There is the need, therefore to carefully study how these practices are carried out by farmers and improvement made upon them where necessary to enhance their effectiveness in fruit fly suppression. Mango is one of the most important tropical fruit crops grown worldwide. Its demand and cultivation are also on the increase worldwide. In the Gamo district, it is the second income generation and providing employment opportunity to a large number of population to the banana. Mango production is also aimed at increasing the food security of the nation by providing suitable fruit that is rich in many of the nutrients required for the proper nourishment of the body. One of the major constraints to the production of this important crop is the attack by arthropod pests, among which the fruit flies and white mango scale are most destructive. Fruit flies generally believed to cause yield losses of up to 30-80% in East Africa and also ranks high among the quarantine pest of fruit and vegetable crops worldwide.

The awareness of Ethiopian farmers of the pest status and current management options for the control of this pest was studied in two districts in the Gamo and Wolaita of Southern Ethiopia. It was found that fruit flies are a major pest infesting mango in Ethiopia. The study also showed that farmers are already aware of the tremendous yield and other losses that can be incurred due to the activities of the pest. Their quarantine status was found to be clearly understood by some farmers. Management methods such as the use of insecticides, cultural control (e.g., destruction of fallen fruits), trapping alone or in various combinations in an IPM approach, are practiced against the pest by farmers in the study area.

V. Recommendations

The results of this survey indicate that there is an information gap between on mango producers in pest status and management strategies on mango insect pests and diseases. Some farmers have do not know fruit flies. Therefore, there are needs of aggressive public advocacy by extension workers, NGOs and other responsible organizations to increase farmer's awareness of fruit flies species and their effect on fruit production. There is the need to study the rate of infestation of fruit flies in farmers' field, to confirm whether there is a displacement of the indigenous species of fruit flies and to check the presence of natural enemies in the mango agro-ecosystem. This will curtail the development of new pest spectrum in other fruit and vegetable crops that may arise from host switching by those displaced species. This will also have implications for control strategies aimed at fruit fly management.

Acknowledgement

Icipe(International Center for Insect Physiology and Ecology) gratefully acknowledged for funding this research.

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Global Journal of Science Frontier Research: D
Agriculture and Veterinary
Volume 20 Issue 1 Version 1.0 Year 2020
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Growth, Pod Yield and Quality of Hot Pepper (C*apsicum Annuum* L.) as Affected by Variety and Rates of Nitrogen Fertilizer in Wolaita, Southern Ethiopia

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Keywords: hot pepper, nitrogen, pod yield, quality, variety.

GJSFR-D Classification: FOR Code: 070199

Strictly as per the compliance and regulations of:

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Keywords: hot pepper, nitrogen, pod yield, quality, variety.

I. Introduction

Hot pepper (*Capsicum annuum* L.) is the world's most important vegetable crop that ranks second after tomato and uses as fresh, dried, vegetable, spices, and condiments (Acquaah, 2004). Hot pepper is a warm season, high-value crop important in the local dishes, *Karia*, *berbere,* and processing industries as a coloring agent and raw material for the export market in the form of oleoresin (Bosland and Votava, 2000; Dessie and Birhanu, 2017).

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Agro-climatic and edaphic conditions of Ethiopia is suitable for production of *Capsicums* in both rain-fed and irrigated conditions (Dessie and Birhanu, 2017). Hot pepper is grown in many parts of Ethiopia, among them Amhara, Oromia, and Southern Nations and Nationality People's Regional States (SNNPRS) are the major ones (Rutgers, 2010). Cultivation of red pepper (180,701.46 ha) and green pepper (9,832.28 ha) achieved 1.83 t ha^{-1} red and 6.3 t ha^{-1} green in Ethiopia (CSA, 2017). Its productivity in research conditions reached 1.8-2.5 t ha^{-1} of dried pepper and 15 - 20 t ha^{-1} green peppers (Lemma *et al.*, 2008). However, the average dry and green yield of hot pepper in small scale farmers is very low compared to the world's average dry and green production (2.2 t ha^{-1}) and (17.8 t ha^{-1}), respectively (FAO, 2016). Oleoresin content of *Capsicums* ranged between 9.0% in 'PBC-776' and 21.8% in 'PBC-380' in (Pandey *et al*., 2008) whereas 3.5% of oleoresin was obtained from Marako Fana variety in Ethiopia which is quite low compared to the international standard (5-12%) (Rutgers, 2010).

Production of improved variety and nutrient management of *Capsicum* are pillars of the improved technologies to robust benefit for producers to achieve sustainable hot pepper production to increase their income and contribute to their livelihood.

Variety is an important factor for successful crop production. An improved genotype can show better growth, higher yield, and quality of hot pepper. However, the limitation brought about by lack of high yielding and well-adapted varieties, inappropriate fertilizer utilization, poor extension services, poor marketing system, and presence of diseases and insect pests resulted in low productivity in the country (Seleshi, 2011). Further, the use of unimproved cultivars significantly affects growth, yield and yield components, and quality of pepper. Dessie and Birhanu (2017) reported that unimproved cultivar Woreta local is used in the Fogera research center gave low yield and poor in quality. The green pod yields obtained from local and Melka Awaze were 5.366 and 14.529 t ha^{-1}, respectively (Dessie and Birhanu, 2017). Similarly, Rutgers (2010)

reported the quality of hot pepper oleoresin extraction affected by cultivars.

According to Alemu and Ermias (2000), low soil fertility is another yield-limiting factor for hot pepper production. Hot pepper requires an adequate amount of major and minor nutrient but nitrogen and phosphorous used dominantly (Bosland and Votava, 2000). Nitrogen is an essential constituent of protein and enzyme, which directly affects several biochemical processes, mainly photosynthetic activity (Marschner, 2012). It influenced the growth and yield of hot pepper production (Ayodele *et al.,* 2015). An adequate amount of N is vital for optimum growth, yield, and quality, but Havlin *et al*. (1999), an excess of N in relation to other nutrients such as P, K, and S, can delay crop maturity. An excessive application of N fertilizer creates pollution of agro-ecosystem and leads to some adverse effects on soil fertility (Fischer and Richter, 1984); developed necrotic lesions followed by defoliation of leaves (Hartz *et al*., 1993); leading to reduced yield and high cost of production (El-Shobaky, 2002).

In general, lack of high yielding varieties and rate of N fertilizer application are major yield-limiting factors (Ayodele *et al.,* 2015; Dessie and Birhanu, (2017). Abrham *et al.* (2017a) studied some of the varieties for growth, yield, and quality in Wolaita, but their suitability with an adequate amount of N fertilizer has not been understood and problems are not well addressed in the study area. Production of hot pepper for growth, high fruit yield, quality, and resistance to pest and diseases in the existing agro-ecology critically needs evaluation, and nitrogen fertilizer recommendation is an issue of priority to utilize the crop potential in the area that at the end will contribute for sustainable pepper production. Thus, the current research was initiated with the following objectives:

- To identify best performing hot pepper variety in terms of growth, pod yield, quality, and disease resistance in the study area.
- To determine the optimum rates of N fertilizer for hot pepper production in the Wolaita area.

II. Materials and Methods

a) Description of the Study Area

The study was conducted at the research site of Wolaita Sodo University, College of Agriculture, and Department of Horticulture during the 2017/18 cropping season. The experimental site is geographically located at 6° 49 'N latitude, 37° 45'E longitude with an altitude of 1886 meter above sea level. The area receives an annual average rain fall of 1520 mm, and the average annual minimum and maximum temperatures are 14°C and 25°C, respectively (Abrham *et al*., 2017b). The type of soil is sandy clay loam with pH 5.9.

b) Experimental Materials, Treatments, and Design

The experiment consisted of four hot pepper varieties, namely Melka Awaze, Melka Shote, Avpp0514, and Avpp0206. Among four varieties, two of them (Melka Awaze and Melka Shote) were obtained from Melkasa Agricultural Research Centre (MARC), and the rest two Avpp0514 and Avpp0206 were introduced varieties from Asian Vegetable Research and Development Center (AVRDC). Four levels of nitrogen 0, 50, 100, and 150 kg N ha^{-1} were used as the second factor. Urea (46% N) was used as source of nitrogen (N) and applied by split application method (half at planting and the remaining half applied 30 days after transplanting). NPS was used as a source of phosphorous and nitrogen. TSP was used as a source of phosphorous. The 250 kg ha^{-1} NPS (19% N, 38% P_2O_5, and 7% S) fertilizer was applied at the time of transplanting.

The experiment was conducted using 4 x 4 factorial combinations (16 treatments), which were laid out in randomized complete block design (RCBD) with three replications. The transplanting was done using spacing of 70 X 30 cm between rows and plants, respectively. Each plot consisted of four rows and ten plants per row with a gross plot size of 2.8 m x 3.0 m (8.40 m^2). All other cultural practices were done as per the recommendation of MARC (EARO, 2004). The detail of the treatment combinations was shown in Table 1.

Table 1: The treatment combinations used during the experiment

Variety	Nitrogen fertilizer (kg ha^{-1})	Treatment (T)	Treatment combination	Variety	Nitrogen fertilizer (kg ha^{-1})	Treatment (T)	Treatment combination
V1	N1	T1	V_1N_1	V3	N1	T9	V_3N_1
	N2	T2	V_1N_2		N2	T10	V_3N_2
	N3	T3	V_1N_3		N3	T11	V_3N_3
	N4	T4	V_1N_4		N4	T12	V_3N_4
V2	N1	T5	V_2N_1	V4	N1	T13	V_4N_1
	N2	T6	V_2N_2		N2	T14	V_4N_2
	N3	T7	V_2N_3		N3	T15	V_4N_3
	N4	T8	V_2N_4		N4	T16	V_4N_4

Where, V_1= Melka Awaze, V_2= Melka Shote, V_3= Avpp0514, V4= Avpp0206, N_1=0 kg N ha^{-1}, N_2= 50 kg N ha^{-1}, N_3= 100 kg N ha^{-1} and N_4=150 kg N ha^{-1}

c) *Data Collected*

In each treatment, ten plants from each plot were randomly selected from the central two rows, and qualitative and quantitative traits were measured as indicated below.

i. *Phenological and growth data*

Days to 50 % flowering: The days recorded when 50% of the plants bear flowers after transplanting.

Days to first fruit set: This was recorded when a plant starts to set the first fruit.

Days to the first harvest: The number of days from transplanting to the date of the first harvest was recorded.

Plant height (cm): The length of the plant was measured from the soil surface to the tip of plants in each plot at plant maturity.

Leaf area (cm^2): Leaf area of the targeted plants was estimated from individual leaf length and leaf width from top, middle and bottom parts of plants and averaged using the formula developed by Erik *et al*. (2004):

$$LA = 0.69 \text{ X } LxW \text{ ---------- (1)}$$

Where, LA= Leaf area, L=Leaf length, W= Leaf width.

Leaf area index (LAI): Is the amount of leaf area (cm^2) in a canopy per unit ground area (cm^2) of plants (Yildirim *et al.,* 2017). The values were obtained by the number of plants and their respective ground area (30 cm x 70 cm).

$$\text{Leaf area index (LAI)} = \frac{Leaf\ \ area\ \ of\ \ a\ plant\ \ (\text{cm}^2)}{Ground\ \ area\ \ of\ \ single\ \ plant\ \ (\text{cm}^2)} \quad (2)$$

ii. *Yield and yield components*

The Number of pods per plant: The number of pods per plant was obtained by counting all fruits produced and divided by the number of sample plants.

Marketable pod numbers per plant: The average number of pods free from diseases, insect pest, and other defects were obtained by counting from sample plants.

Marketable pod yield (t ha^{-1}): Was determined by sorting fruits according to color, shape, size, and free of any mechanical or disease injuries and acceptable by the market.

Total pod yield (t ha^{-1}): The total sum of marketable and unmarketable pod yield of plants measured, and the yields obtained from plots were converted to a hectare base.

iii. *Pod quality*

Pod length (cm): Average pod length measured from tip of the pod to basal end of ten ripe sample pods of the second harvest were measured using venire caliper.

Pod width (cm): Average pod width of ten ripe pods of the second harvest was measured at the widest point of the pods were measured using venire caliper.

Pod wall thicknesses (mm): An average of ten ripe fruits of the second harvest was cut at the middle of the pod, and the pod wall (pericarp) thickness was measured using venire caliper.

Oleoresin content (W/W %): The samples were pods collected from each plot subjected to shade dried, ground to make powder and 10 – 20 g were used for oleoresin extraction and measured by using weight to weight basis in percentage.

Disease incidence

Disease incidence (%): Starting from thirty days after transplanting, the plants were regularly monitored and recorded. The number of infected plants was considered, and the percentage of infected plants with disease incidence was estimated as suggested by Agrios (2005).

$$\text{Disaese Incidence (\%)} = \frac{Total\ \ number\ \ of\ infected\ \ plants}{Total\ \ number\ \ of\ examined\ \ plants} X100\text{----} \text{ ---------- (3)}$$

iv. *Data Analysis*

Analysis of variance

The data were subjected to analysis of variance (ANOVA) of RCBD in factorial arrangements using SAS software (SAS, 2002) version 9.1. All significant mean separation was compared using Least Significant Difference (LSD) test at 5% probability level. Oleoresin content (W/W %) was analyzed by descriptive statistics using the chart.

III. Results and Discussion

a) *Crop Phenology and Growth Traits*

Analysis of variance revealed that the days to 50% flowering had significantly ($P < 0.001$) affected by variety (V) and rates of nitrogen (N) fertilizer, whereas their interaction effect was non-significant (Table 2).

Table 2: Analysis of variance showing mean squares for crop phenology and growth of hot pepper as affected by the interaction of varieties and rates of nitrogen fertilizer in Wolaita, 2017/18

Source of variation	Df	Days to 50% flowering	Days to first fruit	Days to firs harvest	Plant height (cm)	Branch number	Canopy diameter (cm)	Leaf Area (cm^2)	Leaf Area Index
Rep.	2	3.94	4.15	15.02	6.64	1.36	2.55	7.56	171.01
Variety (V)	3	421.09***	564.75***	107.74**	101.45***	5.14**	181.69***	122.58***	2781.52***
Nitrogen (N)	3	1142.24***	1245.25***	601.74***	866.09***	3.31*	472.11***	28.22***	635.48***
V x N	9	21.92^{ns}	34.95^{ns}	16.67^{ns}	13.56*	1.22^{ns}	16.07^{ns}	4.17*	95.54*
Error	30	40.49	41.63	16.20	5.49	0.73	23.19	1.37	29.92

df=degree of freedom; *, **, *** indicate significance at $P < 0.05$, at $P < 0.01$, and at $P < 0.001$, respectively, 'ns' not significant.

The earliest days to 50% flowering (53.33 days) was attained by introduced variety Avpp0514, whereas locally released variety Melka Shote (65.75 days) required the longest days to 50% flowering (Table 3). This variation might be due to inherited differences in variety. This result agrees with that of Amare *et al*. (2013) who reported that maximum number of days for fifty percent of the plants in a plot to flower was taken by variety Melka Zala (99 days) in plots treated with 0 kg N ha^{-1} and 0 kg P_2O_5 ha^{-1} of the fertilizers (control). Seleshi (2011) and Melaku *et al*. (2015) indicated that earliness or lateness in the days to 50% flowering could be affected by inherited characters.

Data presented in Table 3 showed that days to 50% flowering had a range of 22.94 days. The longest day to 50% flowering (73 days) was recorded for treatments received 0 kg N ha^{-1} whereas the earliest (50.06 days) to 50% flowering was recorded for 100 kg N ha^{-1}. This variation might be due to N fertilizer affect positively on flowering initiation, where the early flowering acceleration of the vegetative phase through the cumulative effect of the absorbed nutrients on the photosynthesis process but over dose of nitrogen had delayed time of flowering as result of consumption of metabolites by vegetative tissues. This result was supported by Aminifard *et al.* (2012) who, reported that the N application accelerated the appearance of first flower and plants flowered earlier (45.26) treated at 100 kg N ha^{-1} than control (47.84) and then after that delayed to 46.76 days at 150 kg N ha^{-1}. Similar reports observed in Yamane (2017) in Tigray.

Days to first fruit set was significantly ($P < 0.001$) affected by variety and rates of nitrogen fertilizer, but their interaction effect was non-significant (Table 2).

The result indicated that the earlier days to first fruit set (54.92 days) was attained by introduced variety Avpp0514, whereas locally released variety Melka Shote (68.92 days) required longer time (Table 3). In this study, the result showed that introduced variety Avpp0514 attained 25.49% and 23.04% days earlier to first fruit set than local released variety Melka Shote and Melka Awaze, respectively. In general, introduced variety Avpp0514 and Avpp0206 attained first fruit set earlier than local released varieties Melka Shote and Melka Awaze. This result might be due to the effect of inherited characters of the hot pepper. The result agrees with Tibebu and Bizuayehu (2014) who, reported that locally released variety Marako Fana and Melka Shote was non-significant in days to fruit set observed, but variety Marako Fana attained longest days to first fruit set (95.29 days) than Melka Shote (93 days).

In the case of N rates, the longest days to first fruit set (76 days) was recorded for 0 kg N ha^{-1} whereas the earliest (52 days) to first fruit set was recorded at treatments received 100 kg N ha^{-1} (Table 3). As the rate of N application increases from 0 to 100 kg N ha^{-1}, the number of days taken to first fruit set decreased. However, it increased at 150 kg N ha^{-1}. The effect of over dose nitrogen on the days to flowering and fruit setting increased compared to the optimal dose. Similarly, Aminifard *et al.* (2012) reported that N enhanced vegetative growth and reduced reproductive growth and N application beyond 50 kg ha^{-1} to 150 kg ha^{-1} had a non-significant effect on fruit set. Therefore, an adequate supply of N was economical and essential for better growth and development of hot pepper. Tibebu and Bizuayehu (2014) observed similar results that indicated increasing nitrogen fertilizer up to 150 kg N ha^{-1} increased days to fruiting. In line with Addisalem (2011) reported increasing nitrogen fertilizer up to 150 kg N ha^{-1} increased days to first fruit set from 99.3 to 111.6 days. This result might be because variety was different in response to N fertilizer for the first fruit set.

Table 3: Effect of variety and rates of nitrogen fertilizer on days to 50% flowering, days to first fruit set and first harvest in Wolaita, 2017/18

Treatment	Days to 50% flowering	Days to first fruit set	Days to first harvest
Variety			
Melka Awaze	63.58a	68.92a	85.50bc
Melka Shote	65.75a	67.58a	90.67a
Avpp0514	53.33b	54.92b	83.92c
Avpp0206	56.08b	58.42b	88.33ba
LSD (0.05)	5.30	5.37	3.35
Rate of N (kg ha-1)			
0	73.0a	76.08a	97.00a
50	55.5b	58.50b	80.92c
100	50.06c	52.00c	83.33c
150	60.08b	63.25b	87.17b
LSD (0.05)	5.30	5.37	3.35
CV (%)	10.66	10.3	4.62

LSD (0.05) = Least Significant Difference at 5% level, CV= coefficient of variation, Means in a column followed by the same letters are not significantly different at 5% level of significance

The earliest day to first harvest (83.92) was attained by introduced variety Avpp0514, whereas the longest day to first harvest (90.67 days) was recorded by locally released variety Melka Shote (90.67). The variety of hot pepper response to days to the first harvest might be due to genetic traits and earliness or lateness of days to 50% flowering and days to first fruit set. Similarly, Seleshi (2011) reported that among eight elite hot pepper varieties Melka Shote compared to variety Gojeb local attained highest days to the first harvest by 51.1% in Jimma. On the other hand, hot pepper with 0 kg N ha^{-1} application harvested late (97.00 days) while application of 50 kg N ha^{-1} shown earlier (80.92) days to first harvest. In this study, days to the first harvest hastened with increasing level of N

fertilization from 0 to 50 kg N ha^{-1} then after that delayed N rate increased from 50 kg N ha^{-1} to 150 kg N ha^{-1} (Table 3). The longest days to first harvest in control result is as a result of insufficient N fertilizer at the hot pepper.

Analysis of variance revealed that plant height was significantly ($P < 0.05$) affected by variety, rates of nitrogen, and the interaction effect (Table 2). The longest plant height (77.6 cm) was recorded for introduced variety Avpp0514 with a 100 kg ha^{-1} rate of nitrogen fertilizer whereas the shortest (50.97 cm) plant height was recorded for variety Avpp0206 at 0 kg ha^{-1} nitrogen. In this study, all the varieties of hot pepper showed an increase in plant height with increasing N fertilizer only up to 100 kg ha^{-1} after that at 150 kg ha^{-1} declined in all varieties (Table 4). In this study, the variety was very highly significantly influenced plant height of the crop. This result was in line with the findings of Abrham *et al.* (2017a) that for 19 varieties studied; plant height ranged from 32.78 to 71.0 cm in Wolaita Sodo. This result, also supported by Haileslassie *et al*. (2015) findings on Melka Awaze, recorded the highest plant height (82.0 cm) while Melka Shote recorded the least (54.50 cm). Tibebu and Bizuayehu (2014) also reported that N affected plant height of hot pepper. The increases in plant height with respect to increased N rate might have due to an increase in cell elongation and the maximum vegetative stage of the plant. In line with this result, Aminifard *et al*. (2012) reported that the variation in plant height might be due to the amount of nitrogen fertilizer increased up to 100 kg ha^{-1} and then declined at 150 kg ha^{-1} (28.34 cm).

Table 4: Interaction effect of variety and rates of nitrogen fertilizer on plant height in Wolaita, 2017/18

Variety	Plant height (cm)				Leaf area (cm^2)				Leaf area Index			
	Nitrogen Rate (kg ha^{-1})				Nitrogen Rate (kg ha^{-1})				Nitrogen Rate (kg ha^{-1})			
	0	50	100	150	0	50	100	150	0	50	100	150
Melka Awaze	56.73^{gh}	72.20^{b}	73.73^{ab}	64.93^{de}	15.67^{g}	18.77^{ef}	19.83^{de}	16.67^{fg}	74.70^{g}	89.43^{ef}	94.37^{de}	79.33^{fg}
Melka Shote	52.37^{ij}	66.10^{de}	67.53^{de}	60.37^{fg}	14.93^{g}	18.37^{ef}	19.33^{de}	15.36^{g}	71.00^{g}	87.37^{ef}	92.00^{de}	73.10^{g}
Avpp0514	55.20^{hi}	75.60^{ab}	77.60^{a}	63.90^{ef}	16.80^{fg}	20.40^{c-e}	19.77^{de}	21.33^{cd}	79.90^{fg}	97.20^{c-e}	94.13^{de}	101.53^{cd}
Avpp0206	50.97^{j}	68.17^{cd}	71.93^{bc}	65.37^{de}	22.40^{bc}	24.60^{ab}	24.47^{ab}	25.00^{a}	106.77^{bc}	117.07^{ab}	116.27^{ab}	119.13^{a}
LSD (0.05)	3.90				1.95				9.11			
CV (%)	3.60				5.97				5.86			

LSD (0.05) = Least Significant Difference at 5% level, CV= coefficient of variation, Means in a column followed by the same letters are not significantly different at 5% level of significance

b) Leaf area and leaf area index

ANOVA result indicated that leaf area and leaf area index were significantly ($P < 0.05$) influenced by variety, rates of nitrogen fertilizer, and their interaction effect (Table 2). The highest leaf area (25.00 cm^2) and leaf area index (119.12) were obtained from introduced variety Avvp0206 with the application of 150 kg N ha^{-1} while the lower leaf area (14.93cm^2) and leaf area index (71.00) were achieved at local released variety Melka Shote with the application rate of 0 kg N ha^{-1} (Table 4). This result agreed with Aydole *et al*. (2015) who, reported that nitrogen fertilizer increased from 0 kg N ha^{-1} to 75 kg N ha^{-1} increased leaf area per plant which ranged 23.51 cm^2 to 51.80 cm^2 in "Rodo" variety. Addisalem (2011) supported that application of N at the rate of 150 kg N ha^{-1} increased leaf area and leaf area index by 186.1% and 190.9%, respectively than control. The observed increase in leaf area and leaf area index as a result of the application of N rates to the varieties of pepper might be through its effect on increased vegetative growth.

This could be attributed to the genetic characteristics of hot pepper coupled with N fertilizer increase in leaf area and leaf area index due to applied N rates. Generally, attaining optimum leaf area and leaf area index is necessary to intercept the maximum light energy. The current investigation agreed with Tibebu and Bizuayehu (2014), who confirmed that the leaf area index was significantly affected by N fertilizer. Again Sintayehu *et al.* (2015) also reported that leaf area and leaf area index was significantly influenced by the interaction effects between mulch types and varieties of hot pepper.

c) Yield and Yield Components

i. *Number of pod per plant*

The number of pods per plant was significantly ($P < 0.01$) influenced by variety and rates of nitrogen fertilizer main effects, whereas the interaction effect was non-significant ($P < 0.05$) (Table 5).

Table 5: Analysis of variance showing mean squares for yield and quality traits of hot pepper as affected by the interaction of variety and rates of nitrogen fertilizer in Wolaita, 2017 /18

Source of variation	Df	NPP	MPNP	TPY (t ha^{-1})	MPY (t ha^{-1})	PL	PW	PWT	DI
Replication	2	381.94	199.095	12.00	9.77	115.65	3.65	0.004	2.08
Variety (V)	3	463.23**	429.97***	135.744***	138.59***	1553.33***	94.65***	0.38***	904.69***
Nitrogen (N)	3	1597.17***	1333.35***	129.34***	122.83***	258.96***	34.12***	0.12***	488.02***
V x N	9	104.74^{ns}	80.18^{ns}	9.51*	9.46*	128.25***	3.86***	0.02***	108.39***
Error	30	68.73	62.32	3.07	2.94	20.60	0.50	0.002	7.64

df=degree of freedom; *, **, *** indicate significance at P≤0.05, at P≤ 0.01, and at P ≤ 0.001, respectively, 'ns' not significant; NPP= Number of pod per plant, MPNP= Marketable pod number per plant, TPY= total pod yield, MPY= marketable pod yield, PL= Pod length, PW= Pod width, PWT= Pod wall thickness, DI = Disease incidence

The result indicated that the main effect of introduced variety Avpp0514 showed a highly significant yield advantage over Melka Awaze by 52.86 % in pod number per plant with significant variation between introduced and the locally released one (Table 6). This variation might be due to genetic characteristics of varieties in the production of the highest plant height, the largest leaf area, leaf area index, and the widest canopy diameter. In line with this study, Abraham *et al.* (2016) reported the significant difference among four cultivars of hot pepper in the number of fruits per plant that ranged from 46.2 to 113.2 in 2013 and 35.56 to 53.56 in 2014 at Derashea. Seleshi (2011) showed that wider canopy diameter could produce more fruit (pods) than varieties with narrow canopy in Jimma and Kechema.

Nitrogen treatments affected the total number of pod per plant. Increasing the rate of N from 0 to 50 kg ha^{-1} significantly increased the total pod number per plant. According to the present study, 50 kg N ha^{-1} showed highly significant yield advantage over both extremes (0 kg N ha^{-1} and 150 kg N ha^{-1}) by 114.1% and 50.17%, respectively. This shows the optimum rate of N for an enhanced number of pod production was already reached at 50 kg ha^{-1} and increasing the rate of the nutrient beyond that could have a negative impact on the production of the number of pod per plant. In agreement with these results, Aydole *et al*. (2014) reported significant difference and marketable -pod number ha^{-1} increased from 206.72 to 400.00 x 10^3 in 2010 and 242.75 to 450.98 x 10^3 in 2011with increasing rates of N from 0 to 75 kg ha^{-1}. This is supported by the findings obtained with Aminifrad *et al*. (2012), who reported that increasing N applied to pepper plants from 0 to 100 kg N ha^{-1} was accompanied by the highest fruit number (19.26) for 100 kg N ha^{-1}. In general, over- and under-dose rate of N fertilizer reduced number of pod per plant compared to an optimum rate of N application.

Table 6: Effect of variety and rates of nitrogen fertilizer on pod number per plant in Wolaita, 2017/18

Treatment	NPP	MPN
Variety		
Melka Awaze	28.53^{c}	22.06^{c}
Melka Shote	35.78^{bc}	25.99^{cb}
Avpp0514	43.61^{a}	36.17^{a}
Avpp0206	37.66^{ba}	29.53^{b}
LSD (0.05)	6.91	6.28
Rate of N (kg ha^{-1})		
0	22.44^{c}	15.74^{c}
50	47.44^{a}	38.54^{a}
100	44.11^{a}	35.51^{a}
150	31.59^{b}	23.96^{b}
LSD (0.05)	6.91	6.28
CV (%)	22.77	26.54

LSD (0.05) = Least Significant Difference at 5% level; CV= coefficient of variation. Means in a column followed by the same letters are not significantly different at 5% level of significance; NPP=number of pod per plant MPN= marketable number of pod per plant UMPN=unmarketable pod number per plant

ii. *Marketable pod number per plant*

Variety Avvp0514 gave a significantly higher number of marketable pod numbers (36.17) per plant as compared to the lowest marketable pod number produced by Melka Awaze (22.07) (Table 6). The present study indicated that introduced varieties were superior to the locally released ones. This result was agreed with Awol *et al*. (2011), who reported that there was a significant difference among five varieties in marketable number of pods per plant and which ranged from 14.7 to 25.4. Yemane (2017) also reported that among five released varieties, Melka Shote produced more number of fruits, and it was statistically superior to the others.

The maximum marketable number of pod per plant (38.54) was obtained at the rate of 50 kg N ha^{-1}, whereas the smallest number of pod per plant (15.74) was obtained from 0 kg N ha^{-1}. This shows that the optimum rate of nitrogen for enhanced marketable number of pod per plant was already reached at 50 kg N ha^{-1}. Application of N at the rate of 50 kg ha^{-1} showed a highly significant advantage of marketable pod number per plant over 0 kg N ha^{-1} by 144.85% and by 60.85% over 150 kg N ha^{-1}. Decreasing or increasing the

rate of the N fertilizer beyond the optimum level negatively affected the marketable number of pod per plant. This could be attributed to the early days to fruit set and the first harvest attributed to optimum rates of N, which resulted in a higher number of marketable pods.

iii. *Total pod yield per hectare*

The current investigation revealed that the total pod yield per hectare was highly significantly ($P < 0.001$) affected by variety and rates of nitrogen fertilizer and significantly ($P < 0.05$) by the interaction effect (Table 5). Based on the analysis, the highest total pod yield (16.83 t ha^{-1}) was attained by the introduced variety Avpp0514 coupled with the rate of N at 100 kg ha^{-1} whereas the lowest (2.07 t ha^{-1}) was recorded for Melka Shote at 0 kg N ha^{-1} (Table 7). In this study, the total pod yield of introduced varieties Avpp0514 and Avpp0206 increased with the increasing rate of N up to 100 kg ha^{-1}. This result revealed the significant variation of hot pepper varieties for the rate of N application. Increasing N fertilizer beyond optimum was significantly decreased total pod yield per hectare of all varieties; therefore, the optimum rate of N fertilizer is coupled with genetic traits that might have a better response to total pod yield. Similar results were recorded by Aminifard *et al*. (2012) that significant variation was recorded by increasing N applied up to 100 kg N ha^{-1} accompanied with the highest yield per plant than 150 kg N ha^{-1}. Abrham *et al*. (2017a) reported that among 19 varieties tested in Wolaita area, introduced varieties performed well and out yielded the locally released varieties. According to the report, introduced variety Avpp0514 resulted higher than variety Melaka Shote. This finding is also in line with Seleshi (2011), who reported that nine cultivars of hot peppers had shown significant differences in total pod per plant performance.

iv. *Marketable pod yield*

Numerically the highest marketable pod yield per hectare (16.33 t ha^{-1}) was obtained by Avpp0514 variety at the rate of 100 kg N ha^{-1} whereas lowest marketable pod yield (1.6 t ha^{-1}) was achieved by the variety Melka Shote at the N rate of 0 kg ha^{-1}. Variety Avpp0514 at 100 kg N ha^{-1} showed a highly significant yield advantage over Melaka Shote at 0 kg N ha^{-1} by 920.62%. The present study indicated increasing the rate of N from 0 kg ha^{-1} to 100 kg ha^{-1}, the marketable pod yield in variety Avpp0514, and Avpp0206 increased and then declined when the rate of N increased further to 150 kg N ha^{-1} (Table 7). The decrease in marketable pod yield in response to increasing N fertilizer might be due to over application of N fertilizer, which resulted in a negative response to marketable pod yields. Thus, over and under application of N fertilizer beyond the optimum rate was clearly shown a negative impact on marketable pod yield in all varieties. This agrees with the report by Aliyu (2003) that excess N fertilizer application reduced the number of fruits and yield of hot pepper. Furthermore varieties at Bure upper watershed of the Blue Nile in Northwestern Ethiopia were also shown to differ in their response to N and P (Amare *et al.,* 2013). In this study, the highest amount of marketable pod yield might be due to genetic effect coupled with N response that promotes vegetative growth as a result of which would increase plant height, canopy diameter, leaf area, and that might have contributed for higher pod yield. In line with this study, Mebratu *et al*. (2014) reported that increased marketable yield attributed to the enhanced pod length, pod width and pod wall thickness. In this study application of optimum N, rate responded highest plant height, widest canopy diameter, and leaves with larger leaf areas, were a response to marketable yield per plant. Seleshi (2011) also reported that large canopy width, inherited traits of hot pepper on varieties determine yield potential of hot peppers.

Table 7: Interaction effect of variety and rates of nitrogen fertilizer on total and marketable pod yield in Wolaita, 2017 /18

Variety	Marketable pod yield (t ha^{-1})				Total pod yield (t ha^{-1})			
	Nitrogen Rate (kg ha^{-1})				Nitrogen Rate (kg ha^{-1})			
	0	50	100	150	0	50	100	150
Melka Awaze	2.77^{ij}	8.23^{d-f}	6.77^{e-h}	3.87^{h-j}	3.30^{gh}	8.97^{de}	7.23^{ef}	4.23^{f-h}
Melka Shote	1.60^{j}	7.40^{e-g}	5.60^{f-i}	3.74^{h-j}	2.07^{h}	8.17^{e}	6.37^{e-g}	4.25^{f-h}
Avpp0514	6.20^{e-h}	14.93^{ab}	16.33^{a}	8.70^{de}	6.60^{ef}	15.47^{ab}	16.83^{a}	9.27^{de}
Avpp0206	4.40^{g-j}	11.00^{cd}	12.83^{bc}	11.19^{cd}	4.83f^{-h}	11.57^{cd}	13.47^{bc}	11.68^{cd}
LSD (0.05)		2.86				9.92		
CV (%)		21.86				20.87		

LSD (0.05) = Least Significant Difference at 5% level; CV= coefficient of variation. Means in a column followed by the same letters are not significantly different at 5% level of significance

d) Pod Quality

i. *Pod length*

Pod length was significantly ($P < 0.001$) affected by variety, rates of N fertilizer, and their interaction effect (Table 5). The longest pod length (117.17 mm) was attained by variety Avpp0206 at 150 kg N ha^{-1} followed by variety Avpp0514 (115.03 mm) at 50 kg N ha^{-1} whereas the shortest pod length (80.90 mm) was attained by variety Melka Awaze at 0 kg N ha^{-1}

(the control treatment) (Table 8). Generally, introduced varieties showed relatively better performance in response to applications of N fertilizers at all levels. This difference might be attributed to the superior genetic potential of introduced varieties over that of the locally released ones. The better performance of these varieties also may be associated with better canopy diameter, higher leaf area, and leaf area index. This finding was in line with Amare *et al*. (2013), who reported the highly significant differences in pod length concerning the interaction effects of variety, nitrogen, and phosphorous fertilizers. Similarly, Yayeh (2017) reported that pod length of pepper was influenced significantly by the application of N. According to Amare *et al*. (2013), pod length is directly related to the amount of nutrient taken and the vegetative status of the plant. Russo (2003) also observed a positive relationship between fruit weight and pod size, where fruit weight increased linearly with pod length and pod width.

ii. *Pod width*

The highest pod width (19.60 mm) was attained by the introduced variety Avpp0206 at 100 kg N ha^{-1} followed by the same variety while N applied at 50 kg N ha^{-1} (18.50 mm) whereas the narrowest pod width (9.37 mm) was attained by the locally released variety Melka Shote at 0 kg N ha^{-1} (Table 8). This is in line with the investigation of Addisalem (2011), who reported that increasing nitrogen supply to 100 kg N ha^{-1} resulted in about 74% increase in pod width compared to the control treatment in Merako Fana. Similarly, Amare *et al*. (2013) reported that pod diameter could also be influenced by variety or the nutrient supply in the growing environment. Furthermore, Kassa and Atsbha (2015) reported that among four varieties, Melka Sote showed the lowest diameter (1.013cm) in the 2005/2006 cropping season.

iii. *Pod wall thickness*

The thickest pod wall thickness (1.87 mm) was attained by variety Avpp0206 at 50 kg N ha^{-1} whereas the thinner (1.1 mm) ones were attained by variety Melka Shote at the level of 0 kg N ha^{-1} (Table 8). In general, introduced varieties had better thickness than the local released varieties that could possibly contribute better fresh and dry pod yield. In this result, the variation might be due to genetic characters coupled with N fertilizer effect on pod wall thickness. This was supported by the result of Seleshi (2011) who reported that the variation of fruit pericarp thickness due to assimilate partitioning capacity of crops or due to agro-ecological variations. Furthermore, Abrham *et al*. (2017a) found that among 19 genotypes, pod thickness ranged 0.99- 5.63 mm at Areka due to variation in genotypes.

Table 8: Interaction effect of variety and rates of N fertilizer on pod length, pod width and pod wall thickness in Wolaita, 2017/18

Variety	Rate of N (kg ha^{-1})											
	PL (mm)				PW (mm)				PWT (mm)			
	0	50	100	150	0	50	100	150	0	50	100	150
Melka Awaze	80.90^{h}	83.17^{h}	99.63^{d-f}	88.43^{gh}	14.47^{ef}	17.93^{b}	17.43^{bc}	16.33^{cd}	1.13^{h}	1.3^{fg}	1.33^{e-g}	1.17^{h}
Melka Shote	93.37^{fg}	103.60^{b-d}	94.20^{e-g}	84.27^{h}	9.37^{i}	12.60^{h}	12.80^{fgh}	10.55^{i}	1.1^{h}	1.27^{g}	1.33^{e-g}	1.17^{h}
Avpp0514	102.73^{c-e}	115.03^{a}	113.43^{a}	109.77^{a-c}	14.23^{f}	17.73^{b}	18.27^{ab}	18.30^{ab}	1.47^{c}	1.37^{d-f}	1.43^{cd}	1.30^{fg}
Avpp0206	100.20^{d-f}	111.00^{a-c}	112.07^{ab}	117.17^{a}	15.63^{de}	18.50^{ab}	19.60^{a}	14.05^{fg}	1.47^{c}	1.87^{a}	1.67^{b}	1.40^{c-e}
LSD (0.05)	7.56				1.18				0.08			
CV (%)	4.51				4.56				3.26			

LSD (0.05) = Least Significant Difference at 5% level, CV= coefficient of variation, Means in a column followed by the same letters are not significantly different at 5% level of significance, PL=pod length, PW pod width, PWT= pod wall thickness

e) Oleoresin concentration

The result indicated that among 16 treatment combinations, the mean highest (25.89%) oleoresin content was extracted from Avpp0514 variety at 50 kg N ha^{-1} followed by Avpp0514 at 150 kg N ha^{-1} while the mean lowest (13.78%) oleoresin content was obtained from variety Melka Awaze at 0 N kg ha^{-1} (Fig. 1). The result further revealed that over all oleoresin performance of introduced varieties were superior to local ones. In agreement with the current investigation, Pandey *et al.* (2008) reported that among 21 cultivars, oleoresin content varied from 9.0 to 21.8%, which was lower compared to variety Avpp0514 at the rate of 50 kg N ha^{-1}. Introduced variety Avpp0514 at 50 kg N ha^{-1} attained 87.88 % higher in oleoresin content than local released Melka Awaze at 0 kg N ha^{-1} (Annex Table 1). This variation was due to genetic difference of hot pepper. This result also supported by Esayas *et al.* (2011), who reported that Ethiopian varieties exhibited lower values in moisture, protein, fat (oleoresin), and carbohydrate. Therefore, varieties diversification is found to be an alternative option to improve the oleoresin content for the export market in hop pepper production because it is an important quality parameter for export, industrial, and pharmaceutical purposes.

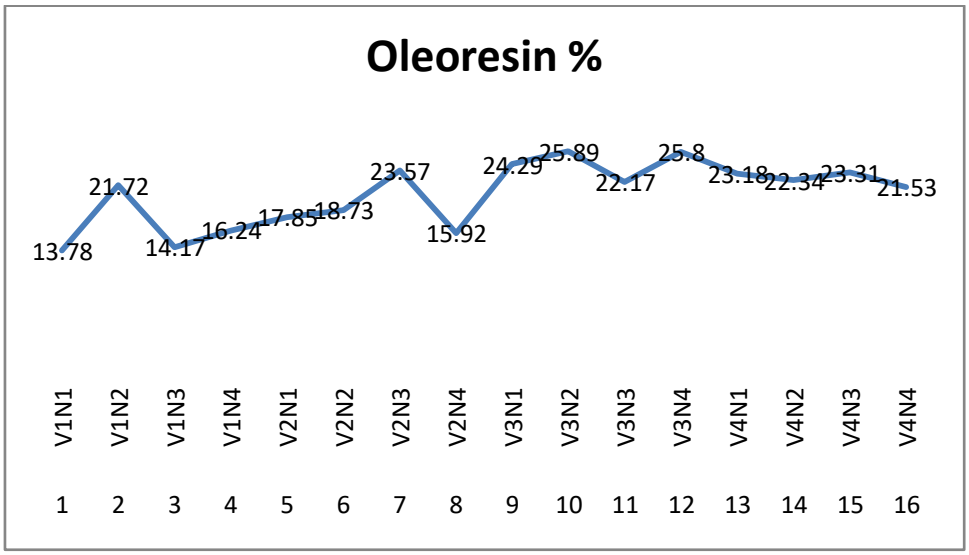

Fig. 1: Mean value of oleoresin contents of hot pepper

f) Disease Incidence

The analysis of variance indicated that there was significant ($P < 0.001$) difference for disease incidence among treatments due to variety, rates of N fertilizer, and their interaction (Table 5). The highest (40 %) of disease incidence was observed by the introduced variety Avpp0206 at 150 kg N ha^{-1} whereas the lowest (1.67 %) percentage of disease incidence was observed by varieties Avpp0514, Melka Awaze and Melka Shote at 0 kg N ha^{-1} and 50 kg N ha^{-1} (Table 9). Further, the result revealed that the magnitude of disease incidence increased as the level of nitrogen beyond optimum in all the tested varieties, but the incidence was almost the same at N levels 0 and 50 kg N ha^{-1}. This might be due to the succulent growth nature at higher N level might have contributed to high disease incidence. In almost all cases, the disease observed was possibly fungal and bacterial. However, introduced variety Avpp0514 was found to be competent with local released varieties in disease tolerance with better yield and quality advantage. This result indicated the response of varieties to disease reaction and the effect of N- fertilizer rates had a significant variation for yield, quality, and disease incidence that could be attributed to the genetic potential of specific variety and the growing environmental conditions. In agreement with the current findings, Addisalem (2011) also reported the least number of sun-scalded pods was obtained at the highest levels of nitrogen. Fungal (*Fusarium* wilt and powdery mildew) and bacterial (wilt, leaf spot, and soft spot) and virus diseases of hot pepper as observed in southern Ethiopia (Shiferewu and Alemayehu 2014). Yemane (2017) reported that among five released hot pepper varieties, Melka Awaze and Melka Shote varieties are the most outstanding ones due to their highest biomass and disease tolerance, which leads to high yield per hectare. Therefore, the use of the best variety, optimum rates of nutrient application coupled with recommended cultural practice were found to be the most important component of integrated pest and diseases management options for hot pepper production.

Table 9: Interaction effect of variety and rates of nitrogen fertilizer on disease incidence in Wolaita, 2017/18

Variety	Disease incidence (%)			
	Nitrogen Rate (kg ha^{-1})			
	0	50	100	150
Melka Awaze	1.67^{g}	1.67^{g}	5.0^{e-g}	8.33^{c-e}
Melka Shote	1.67^{g}	1.67^{g}	5.0^{e-g}	8.33^{c-e}
Avpp0514	1.67^{g}	1.67^{g}	5.0^{e-g}	10.0^{cd}
Avpp0206	6.67^{de}	11.67^{c}	28.33^{b}	40.0^{a}
LSD (0.05)		4.60		
CV (%)		31.97		

LSD (0.05) = Least Significant Difference at 5% level, CV= coefficient of variation, Means in a column followed by the same letters are not significantly different at 5% level of significance

IV. Conclusion

It could be generalized that hot pepper varieties responded differently for variable rates of N– fertilizers in terms of yield, quality, and disease incidence. According to the current investigation, the highest yield advantage was attained by using the introduced variety Avpp0514 with N- rates of 50 kg ha^{-1}. Hence, 50 kg N ha^{-1} was found to be an optimum and efficient fertilization rate for hot pepper growing farmers in the Wolaita area. It could be recommended that diversifying varieties for hot pepper production improves yield obtained per unit area in terms of quality, quantity, and disease reaction.

Acknowledgement

The authors would like to thank Wolaita Sodo University for the financial support to carry out the experiment and also we acknowledge the college of

agriculture, department of horticulture for unreserved support in field and laboratory works that significantly contributed for the successful accomplishment of this research.

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Annex

Table 1: Results of laboratory analysis of oleoresin contents of hot pepper evaluated in Wolaita, Southern Ethiopia in 2017/18

No.	Treatment	Oleoresin %
1	V_1N_1	13.78
2	V_1N_2	21.72
3	V_1N_3	14.17
4	V_1N_4	16.24
5	V_2N_1	17.85
6	V_2N_2	18.73
7	V_2N_3	23.57
8	V_2N_4	15.92
9	V_3N_1	24.29
10	V_3N_2	25.89
11	V_3N_3	22.17
12	V_3N_4	25.8
13	V_4N_1	23.18
14	V_4N_2	22.34
15	V_4N_3	23.31
16	V_4N_4	21.53

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7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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ISSN 9755896