"QUANTIFICATION OF PHYSIOLOGICAL LOSSES DUE TO CORN LEAF APHID, *Rhopalosiphum maidis* (Fitch) IN SORGHUM (*Sorghum bicolor* (L.) Moench)"

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B.Sc. (Ag)

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(ENTOMOLOGY)

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BY

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THESIS SUBMITTED TO THE PROFESSOR JAYASHANKAR TELANGANA STATE AGRICULTURAL UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

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2016

DECLARATION

I**, Ms. ANJALI M S,** hereby declare that the thesis entitled **"QUANTIFICATION OF PHYSIOLOGICAL LOSSES DUE TO CORN LEAF APHID, *Rhopalosiphum maidis* (Fitch) IN SORGHUM (*Sorghum bicolor* (L.) Moench)"** submitted to the **Professor Jayashankar Telangana State Agricultural University** for the degree of **Master of Science in Agriculture** is a result of original research work done by me. I also declare that no material contained in the thesis has been published earlier in any manner.

Place: Hyderabad **(ANJALI M S)**

Date: **I. D. No. RAM/2014-36**

CERTIFICATE

Ms. ANJALI M S has satisfactorily prosecuted the course of research and that the thesis entitled **"QUANTIFICATION OF PHYSIOLOGICAL LOSSES DUE TO CORN LEAF APHID, *Rhopalosiphum maidis* (Fitch) IN SORGHUM (*Sorghum bicolor* (L.) Moench)"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by her for a degree of any University.

Date: **(Dr. D. SRIDEVI)**

Chairperson

CERTIFICATE

This is to certify that the thesis entitled **"QUANTIFICATION OF PHYSIOLOGICAL LOSSES DUE TO CORN LEAF APHID, *Rhopalosiphum maidis* (Fitch) IN SORGHUM (*Sorghum bicolor* (L.) Moench)"** submitted in partial fulfillment of the requirements for the degree of **'Master of Science in Agriculture'** of the **Professor Jayashankar Telangana State Agricultural University**, Rajendranagar, Hyderabad is a record of the bonafide original research work carried out by **Ms. ANJALI M S,** under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all the assistance received during the course of investigations have been duly acknowledged by the author of the thesis.

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Date: **(ANJALI M S)**

Place: Hyderabad

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LIST OF SYMBOLS AND ABBREVIATIONS

%	: Per cent
@	: At the rate of
±	: Plus or minus
>	: Greater than
µl	: Microlitre
µg	: Microgram
µm	: Micrometre
µmol	: Micromole
^{o}C	: Degree Celsius
chl	: Chlorophyll
cm	: Centimetre
dia	: Diametre
df	: Degrees of Freedom
dx	: The number dying within the age group stated in column x
et al.	: and other people
Fm	: Maximum Fluorescence
Fv	: Variable Fluorescence
Fig.	: Figure
g	: Gram
h	: Hour
i.e	: that is
IPM	: Integrated Pest Management

lx	: The number surviving at the beginning of age class noted in column x
lxmx	: Total number of female births in each age interval x
mg	: Milligram
ml	: Millilitre
mm	: Milimetre
mPa	: Mega pascal
mx	: Age-specific fecundity column
No.	: Number
100 qx	: The mortality rate per 100 alive at the start of age interval x
r	: Correlation Co-efficient
r_c	: Capacity for Increase
r_m	: Intrinsic Rate of Increase
rpm	: Rotations per Minute
R_0	: Net Reproductive Rate
RE	: Rutin Equivalent
RH	: Relative Humidity
RWC	: Relative Water Content
SEM	: Standard Error of Mean
SPAD	: Soil Plant Analytical Development
T	: Generation Time
T_c	: Mean or Approximate Generation Time
x	: Age Group or Stage of the development of the insect.
vis-a-vis	: in relation to
viz.	: Namely

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ABSTRACT

The present studies were conducted in the Entomology laboratory and green house, CRIDA, Santhoshnagar, Hyderabad with an objective to study the biology of aphid, to establish relationship between insect density and leaf pigments and estimate the relative water content (RWC) in sorghum due to feeding by corn leaf aphid, *Rhopalosiphum maidis* (Fitch).

R. maidis on sorghum had a life cycle of 7.79±0.12 days, adult longevity of 12.27±0.27 days, life span of 16.25±0.80 days, fecundity and rate of reproduction 35.97 progenies/female, 9.15 progenies/female/day, net reproduction rate (R_0) 45.05, mean generation time (T_c) 9.24, intrinsic rate of natural increase (r_m) 0.44, innate capacity of increase (r_c) 0.41 and corrected mean generation time T 8.73 days, respectively. The length and width of different stages of *R. maidis* were: 720.57± 9.95 µm and 303.62 ± 5.59 µm (1^{st} instar); 978.46 ± 13.51 µm and 412.83 ± 5.53 µm (2^{nd} instar); 1399.52 ±

24.56 µm and 581.74 ± 12.33 µm (3rd instar); 1737.5 ± 20.28 µm and 733.98 ± 10.96 µm (4th instar) and 1833.63 ± 15.89 µm in length and 783.26 ± 10.82 µm (adult), respectively.

The reduction in chlorophyll content, fluorescence and photosynthetic rate ranged between 9.06 to 29.79%; 1.35 to 6.75 (Fv/Fm) and 6.15 to 32.30 µmol CO_2 m^{-2} sec^{-1} respectively, at different levels of infestation, all the three physiological parametes being significantly low at high infestation (300 aphids/plant) compared to no, low (100 aphids/plant) and medium infestation (200 aphids/plant).

A negative correlation between aphid density and physiological parameters indicated that an increase in the incidence of *R. maidis* (density) decreased the chlorophyll content, fluorescence and photosynthetic rate from 20 days after realease of a mixture of aphid population. A significant reduction in all the three physiological parameters were recorded at high density level of aphids on sorghum.

The quantification of leaf pigments *viz.*, chl *a*, chl *b*, chl (*a*+*b*), carotenoid and flavonoid indicated a significant loss in chl (*a*+*b*) and carotenoid in medium and high infestation compared to control. The per cent reduction of these pigments ranged between 17.07 to 39.02 and 10 to 40, respectively at different levels of infestation. However, loss of chl *a* and chl *b* was more significant in medium infestation compared to low and high infestation. On the contrary, the flavonoid content increased significantly in medium and high infestation.

The RWC ranged between 89.3±1.20 to 87.2±1.21%, the per cent of reduction varying between 5.03 to 2.31 at different levels of infestation. The pigment loss was greater at high densities than the water loss.

CHAPTER I

INTRODUCTION

Chapter I

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is an important cereal grain for human and animal consumption throughout the world, and world's fifth most important cereal crop after maize, rice, wheat and barley. It is the dietary staple of more than 500 million people in more than 30 countries with adequate crude protein (8-12%) and high amount of carbohydrates (65-80%). It offers a good substitute for wheat and maize due to its availability, low price and limited human consumption (Etuk, 2012). Nearly 150 insect species have been reported to damage the crop worldwide, causing an estimated loss of over US$ 1000 million annually. Of these, shoot fly, stem borers, greenbug, aphids, shoot bug, spider mites, armyworms, midge, head bug and head caterpillars are the major pests. Several species of aphids can attack sorghum like corn leaf aphid, *Rhopalosiphum maidis* (Fitch), greenbug, *Schizaphis graminum* (Rondani), yellow sugarcane aphid, *Sipha flava* (Forbes), and sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Sharma,1997).

Aphids are an important group of insects with worldwide distribution. They are highly specialized phloem-feeding hemipterans, coevolved multi-level adaptations facilitating exploitation of the resources provided by their host plants (Czerniewicz *et al*., 2011 and Chrzanowski *et al*., 2012). These insects cause a wide spectrum of detrimental effects in infested plant parts, including mechanical disruption of penetrated tissues, depletion of photo assimilates and intensification of many intracellular processes (Sempruch *et al*., 2010 and Sytykiewicz *et al*., 2011). Aphid salivary secretions contain a variety of hydrolytic enzymes and biologically active substances that modulate metabolic reactions within the host. In some cases, aphid infestation contributes to the activation of premature senescing process and programmed cell death in plant organs (Carolan *et al*., 2009 and Anstead *et al*., 2010). Colonization of the different host systems may lead to transmission of a wide range of pathogenic plant viruses (Makkouk and Kumari, 2009).

R. maidis shows a preference for corn, sorghum, and barley. It also infests broomcorn, sugarcane, Sudan grass and many other wild and cultivated grasses, and in all it attacks 26 plant species and 14 species of weeds (Mohamed *et al.*, 2005).The first recorded observation of the corn leaf aphid with maize as host dates back to 1856 by Fitch. The scientific name of the insect was recorded as *Aphis maidis* Fitch and remained for about 100 years. In the year 1947, Etghegaray stated that Webster changed the genus to *Rhopalosiphum maidis* in 1887.

The adult forms of the corn leaf aphid can be found as a winged female (alate), wingless female (apterae) and extremely rare male. The wingless adult is oval, soft-bodied, 2.5 mm long and usually pale bluish-green with black antennae, legs and cornicles. Cornicles have a dark area around the base. The head is marked with two longitudinal dark bands and the abdomen with row of black spots on each side. The nymphs are similar to the wingless adult but smaller and without wings.

Chlorophyll (chl) content is one of the most important parameters in the relationships between plants and herbivores. Chlorophyll levels change during plant development (Costa *et al*., 2001), and can alter in response to a wide variety of stresses (Lawson *et al*., 2001). Chlorophyll content can be reduced by insect feeding, nutritional deficiencies and pathogen infections (Ni *et al*., 2002). Chlorophyll loss caused by herbivore feeding is not fully understood, although herbivory-caused chlorophyll loss has been described (Ni *et al*., 2002 and Heng-Moss *et al*., 2003).

Changes in the total chlorophyll (Chl $a+b$) of foliar tissues is an important indicator of disturbed chloroplast development and impaired photosynthetic capacity in plants exposed to a broad spectrum of biotic and abiotic stresses. Many studies have shown that aphid infestation can trigger severe chlorophyll breakdown within the host. An imbalance between biosynthesis and catabolic turnover of green pigments in plant tissues indicates profound inhibition of photosynthesis process (Heng-Moss *et al*., 2003). Portable leaf-clip-type chlorophyll meters perform rapid, repetitive and nondestructive estimates of chlorophyll concentrations in living plants (Shrestha *et al*., 2012 and Ghosh *et al*., 2013). The use of these reliable and advanced instruments for measurement of green pigment content is not restricted to controlled laboratory bioassays but can be extended to experiments on the plants in natural environment

(Ruiz-Espinoza *et al*., 2010 and Cerovic *et al*., 2012). SPAD-502 chlorophyll meters have been used to obtain accurate measurements in arbitrary SPAD units reflecting absolute foliar chlorophyll concentrations (Shrestha *et al*., 2012). A significant linear relationship between SPAD readings and leaf nitrogen status in tissues of monocotyledonous species have been documented (Rostami *et al*., 2008 and Swain and Sandip, 2010). The emperical relationship established between aphid infestation density and leaf pigments in this study could be very useful for creating pest subroutines in crop modeling studies in future.

Aphid feeding decreases the moisture content of infested leaves. Plants under the stress of early-season infestation allocated more resources for leaf growth, but stem growth was severely retarded (Petitt and Smilowitz, 1982). Herbivorous insects in soybean resulted in water loss in the infested leaves (Aldea *et al*., 2005).

In the light of the above information, the present studies were contemplated with the following objectives, as the physiological studies relating to *R. maidis* on sorghum are limited.

OBJECTIVES:

1. To study the biology of the corn leaf aphid, *R. maidis* on sorghum.
2. To establish the relationship between insect density and leaf pigments.
3. To estimate the relative water content (RWC) in sorghum due to aphid feeding.

CHAPTER II

REVIEW OF LITERATURE

Chapter II

REVIEW OF LITERATURE

Sorghum suffers from aphid infestation which causes deleterious effects on the physiological processes resulting in reduction in growth and losses in yield. With this background, the review summarizes the results from several years of research on relationship between insect density and leaf pigments and relative water content (RWC) in sorghum due to aphid feeding.

2.1 BIOLOGY OF THE APHID

Juan and Frank (1964) reared *Rhopalasiphum padi* (L.) at 13, 23, 26, and 30^{o}C. The average nymphal production per female was 30, 42, 50, and 10.9, respectively and the time for a complete generation varied from 21.7 days at 13^{o}C to 5.2 days at 26^{o}C. The lower temperature limit for development was approximately 8^{o}C while the upper limit was about 27^{o}C. Twenty-eight species of Gramineae were tested and most were found suitable as hosts of *R. padi.*

El-Ibrashy *et al.* (1972) observed that the biology of *R. maidis* was affected considerably by the temperature, the host plant, and the physiological age of food plant. The optimum temperature was 30^{o}C; here nymphal development was accelerated, the whole life-span was drastically shortened to less than half than that at 15^{o} and natality rate/surviving female increased with rather negligible deaths among progeny. Barley was the most favourable host and the aphid had about 50 generations/year when they were kept feeding continuously on 5 days old barley plants.

Foott (1977) reported that only small numbers of alatae corn leaf aphids initiated infestations on corn and the large variation in size of aphid infestations observed at pollination was due to differences in the longevity and fecundity of these few early attackers. The whorl leaves which enclosed the tassel before pollination provided a very favourable environment for rapid development of the aphids. Trapping of alatae in yellow pans of water at the periphery of a corn field showed that a minimum temperature of 13^{o}C was required for flight. At a constant temperature of 25.5^{o}C and a

light: dark photoperiod of 14:10 h, the average pre reproductive, reproductive, and post reproductive periods for 29 aphids were 5.9, 15.8, and 9.6 days, respectively. The average number of nymphs produced/female was 68.2.

Cheng *et al.* (2000) in their studies revealed that the survival of sugarcane wolly aphid, *Ceratovacuna lanigera* Zehntner was higher on two to four months old plants. The nymphal stage of the aphid was 15.8 to 16.5 days and adults lived for 20.5 to 24.1 days. Most adult females produced three to five off-springs daily until death, producing an average of 41.0 to 56.6 offsprings in life time.

Beatriz and Alberto (2005) constructed the age-specific life tables of the lettuce aphid, *Nasonovia ribisnigri* (Mosley), feeding on lettuce at different constant temperatures under controlled conditions. Most aphids needed four instars to reach adult stage, but at 8, 26, and 28^{o}C, many individuals passed through five or six molts. Age-specific survivorship (lx) was always above 90% at the temperature range of 16-24^{o}C. Mortality rate (qx) was rather low but constant at 8^{o}C. However, mortality was high at 28^{o}C and occurred mainly in the last nymphal instars and adult stage. No nymphs were produced by the adult morphs at 28^{o}C, but effective fecundity was high at 8^{o}C. Fecundity for alates was always lower than for the apterous aphids at the same temperature. The largest intrinsic rate of natural increase (r_m), and the mean relative growth rate (RGR) occurred at 24^{o}C, for both apterous and alate morphs, and the lowest at 8^{o}C.

Mei *et al.* (2006) conducted experiment on effects of temperature on the life history traits of *R. maidis* at 6, 10, 15, 20, 25, 30 and 35±1^{o}C on corn leaves. At 35^{o}C, only a few nymphs survived and completed development, but all failed to reproduce. The average adult fecundity was as high as 45 offsprings at 15 and 20^{o}C, but dropped to 1.8 at 6^{o}C and 8.6 at 30^{o}C. In general, as temperatures increased, age-specific survivorship (lx) declined more quickly, but age-specific fecundity (mx) peaked earlier. At 25^{o}C, the age-specific net mortality (v_x = lxmx) was the highest in the early reproductive period resulting in the highest intrinsic rates of increase (r_m= 0.329). At 20-30^{o}C, the values of r_m were significantly higher than those at lower temperatures. The results indicated that corn leaf aphids probably were better adapted in population growth to a wider range of high temperatures in warm regions.

Auad *et al.* (2009) evaluated the temperature impact on 12 h old nymph of *R. padi*. Development of *R. padi* was faster with increased temperature, but they did not complete the last nymphal instar at 32^{o}C. The highest fecundity rates were between 16^{o}C and 24^{o}C. The highest fertility (4 nymphs/female/day) was recorded at 12^{o}C and 20^{o}C. The finite rate of increase (λ = 1.9 nymphs/female/day) and the intrinsic rate of increase (r_m = 0.64) were greatest at 24^{o}C and 28^{o}C, respectively.

El-Sheikh *et al.* (2009) studied the biology of *R. maidis* at 15, 20, 25 and 29^{o}C on barley seedlings. The longest life cycle duration (11.7±2.1days) was recorded at 15^{o}C. This duration was decreased to 6.0± 0.7, 5.0± 1.6 and 5.1± 0.9 d at 20, 25 and 29^{o}C, respectively. The fecundity rate was least (7.07 progenies/female) recorded at 15^{o}C. The highest rate (47.18 progenies/female) was recorded at 20^{o}C which gradually decreased to 28.31 and 20.22 progeny/female at 25 and 29^{o}C, respectively. The longest life span duration of 25.9±7.4 days was recorded at 20^{o}C which reduced to 19.5±2.3 days at 15^{o}C, 16.9±3.2 and 14.9±2.8 days at 25 and 29^{o}C, respectively.

Erol (2009) studied the development, survival rate, reproduction and biological parameters of the corn leaf aphid, *R. maidis*, on five corn cultivars at 25^{o}C under laboratory conditions. The corn leaf aphid had a nymphal developmental time of 4.99, 4.98, 4.73, 4.46, and 5.60 days on Ada 9516, K. Arifiye, Primer G626, Pegaso and TTM 815, respectively. The corn leaf aphid reared on K. Arifiye had the highest fecundity (69.65 offspring/aphid). The percentage survival rate, net reproductive rate (R_0) and intrinsic rate of increase (r_m) were lower on TTM 815 than that of other corn cultivars, showing resistance to the pest.

Claudia and Nancy (2010) calculated the life history and some demographic parameters for *Chaetosiphon fragaefoli* (Cockerell) on strawberry. The mean duration of nymphal stages was 10.44 days, the oviposition period was 11.8 days, and the mean number of nymph/female/day was 2.4±0.3. Demographic parameters analyzed included the net reproductive rate R_0 = 14.55±0.096 nymph/female, generation time T=16.91±0.035 days, and the intrinsic rate of increase r_m = 0.158±0.004. No parasites were found associated with *C. fragaefolli*. The pathogenic fungus, *Entomophthora planchoniana* Cornu was the main mortality factor.

Lilian and Carolina (2011) studied the effect of different host plants, including wheat, triticale, forage barley, beer barley, oat and rye on biological parameters of *R. padi* in the laboratory at 24±1^{o}C, 65±10% RH and a 14:10 photoperiod. The results indicated that barley might be the most suitable food for *R. padi* due to greater adult longevity (20.88 days), higher fecundity (41 nymphs/female), higher intrinsic rate of natural increase (0.309 nymphs/female/day), lower doubling time (2.24), and lower nymphal mortality (22.2%) indicating that *R. padi* prefers beer barley for fast and healthy development over other cereal crops.

Araujo *et al*. (2013) studied the life cycle of *Aphis forbesi* Weed on the leaves of the Albion strawberry cultivar at 25±2^{o}C, 60±10% RH and a 12 h photophase. A mean of 1.43 nymphs per female per day were produced. The mean reproductive period was seven days and the mean longevity was 10 days. In every 11 days there was a generation of *A. forbesi*, where each female had the potential to produce between 6 to 9 individuals daily, increasing its population by 1.2 times. The average life cycle was 16.8 days.

Meenakshi *et al.* (2014) observed that a single female of *Brevicoryne brassicae* Linnaeus on cabbage gave birth to 85.8±1.08 nymphs on an average, within its life span. Nymphs developed through four instars and transformed into adults with an average of 8.3±0.83 days. Adult females (wingless) were dull green to grey coloured and covered with a whitish waxy powder. The average pre-natal, natal and post natal periods were recorded as 3.2±0.63, 16.1±4.09 and 10.7±1.16 days, respectively. The average female adult longevity was recorded as 25.6±4.60 days. The incidence of this aphid varied from 20 to 410/3 leaves/plant.

Ruchika and Dolly (2015) studied the biology of the *Aphis gossypii* Glover on cotton under laboratory conditions. The results on different biological parameters showed that the total life duration of female ranged from 28-44 days. The fecundity rate was 1499±458.2 days. The longevity of female aphid on an average was 8.1 days with a maximum of 22 days.

2.2 RELATIONSHIP BETWEEN INSECT DENSITY AND LEAF PIGMENTS

Plant entries that previously tested resistant or susceptible to Russian wheat aphid, *Dillraphis loxia* (Mordvilko) were used to evaluate the effect of aphid feeding on leaf chlorophyll content and *in vivo* chlorophyll fluorescence induction kinetics (John *et al*., 1996). The results suggested that *D. loxia* damage goes beyond the simple removal of photosynthates from the plant. The substantial decrease in Fv/Fm [variable fluorescence (Fv*)* and maximal fluorescence (Fm)] following aphid infestation for the susceptible wheat and barley indicated a significant decrease in the capacity and efficiency of the primary photochemistry of photosystem II.

Walter *et al.* (1999) conducted greenhouse study to characterize leaf reflectance spectra of wheat damaged by Russian wheat aphids (*Diuraphis noxia* Mordvilko) and greenbugs (*S. graminum*) and to determine those leaf reflectance wavelengths that were most responsive to crop stress imposed by these aphid pests. When compared with the control, greenbug feeding damage caused general necrosis in oldest (first) leaves and dramatically lowered the dry weight, leaf area, and chlorophyll concentration of the second, third, and fourth leaves. Russian wheat aphid feeding resulted in a reduction in leaf dry weight and area in the third and fourth leaves, and a reduction in total chlorophyll concentration in all leaves. Leaf reflectance in the 625 to 635 nm and the 680 to 695 nm ranges, as well as the normalized total pigment to chlorophyll *a* ratio index (NPCI), were significantly correlated with total chlorophyll concentrations in both greenbug and Russian wheat aphid damaged plants, indicating that both the wavelength ranges, as well as reflectance index, were good indicators of chlorophyll loss and leaf senescence caused by the aphid feeding damage.

Ni *et al.* (2002) studied the concentration of photosynthetic pigments (*i.e.,* chlorophylls *a* and *b*, and carotenoids) and chlorophyll degradation enzyme (*i.e.,* chlorophyllase, oxidative bleaching, and Mg-dechelatase) activities in aphid-damaged and non-damaged regions of the infested leaves with two infestation periods (6 and 12 days). Russian wheat aphid, *D. noxia* feeding caused significant losses of chlorophyll *a* and *b* and carotenoids in the damaged regions. However, bird cherry-oat aphid, *R. padi* feeding did not, except a significantly lower level of carotenoids in the damaged regions

from the short-infestation (6-day) samples. Interestingly, the non-damaged regions of *D. noxia* infested leaves on both sampling dates had a significant increase of chlorophylls *a* and *b* and carotenoid concentrations when compared with the uninfested leaves. Although *D. noxia* feeding did not cause any changes in either chlorophyll *a/b* or chlorophyll (*a*+*b*)/carotenoid ratio between the damaged and non-damaged leaf regions on short-infestation (6-day) samples, a significantly lower chlorophyll *a/b* ratio was detected in long-infestation (12-day) samples.

Heng-Moss *et al*. (2003) studied Russian wheat aphid, *D. noxia* feeding injury on 'Betta' wheat isolines with the *Dn1* and *Dn2* genes by assessing chlorophyll and carotenoid concentrations, and aphid fecundity. The resistant Betta isolines supported similar numbers of aphids, but had significantly fewer than the susceptible Betta wheat, indicating that these lines are resistant to aphid feeding. *D. noxia* feeding resulted in different responses in total chlorophyll and carotenoid concentrations among the Betta wheat isolines. The infested Betta-*Dn2* plants had higher levels of chlorophylls and carotenoids in comparison with uninfested plants. In contrast, infested Betta-*Dn1* plants had the same level of chlorophyll and carotenoid in comparison with uninfested plants.

Tao *et al.* (2004) studied the plant and aphid biomass, photosynthetic pigment (chlorophylls *a* and *b* and carotenoids) concentrations, and chlorophyll *a/b* and chlorophyll/carotenoid ratios in aphid-infested 'Tugela' near-isogenic lines. Biomass of bird cherry-oat aphid, R. *padi* infested plants was lower than Russian wheat aphid, *D. noxia* infested plants. Concentrations of chlorophylls *a* and *b* and carotenoids were significantly lower in *D. noxia* infested plants compared with R. *padi*-infested and uninfested plants. There was no difference in chlorophyll *a/b* or chlorophyll/carotenoid ratio among Tugela lines. The study demonstrated that *Dn* genes in the Tugela isolines conferred resistance to *D. noxia* but not to *R. padi*. Tugela-*Dn1* was antibiotic, Tugela-*Dn2* was tolerant and antibiotic, and Tugela-*Dn5* was moderately antibiotic.

Ademir *et al.* (2006) determined the damage levels caused by scale insect, *Orthezia praelonga* Douglas (Hemiptera: Ortheziidae) and coffee leaf miner, *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) on rangpur lime and Obata coffee leaves, respectively using infrared analyzer. A negative correlation between infestation level and photosynthesis was found, where the negative inflexion point of the curve was

considered as a reference for damage levels. The control level for *O. praelonga* was below the 7-13% limit for damaged leaf area (40 to 70 scales per leaf), while for *L. coffeella* it was below the 26-36% limit for the same variable.

Chlorophyll loss by soybean aphid, *Aphis glycines* Matsumura was studied in no-choice tests on the infested and uninfested leaves of a susceptible check (KS4202). The minimum combined number of days and aphids needed to detect significant chlorophyll loss was 30 aphids confined for 10 days (John *et al.* 2007). In a similar experiment, seven resistant entries and two susceptible checks indicated the percentage of loss of chlorophyll in the susceptible checks as 40%.

Tulio *et al*. (2009) studied the impact of feeding injury by the Russian wheat aphid, *D. noxia* and bird cherry-oat aphid, *R. padi* on susceptible and resistant wheat by assessing photosynthetic parameters. Both aphids negatively affected net photosynthesis, *D. noxia* had a greater impact than *R. padi*, even when aphid numbers were considerably fewer for *D. noxia* (100-150 aphids per plant) compared with *R. padi* (>200 aphids per plant). The photosynthetic pigment and carbohydrate data suggested that the initial net photosynthesis reduction elicited by aphid feeding may not be directly related to the light reaction portion of the photosynthetic pathway via pigment losses. Also it was unlikely that source-sink manipulation was the primary cause for the observed short-term inhibition of photosynthesis.

Sylwia *et al*. (2010) determined chlorophyll *a* and *b* levels (SPAD readings) in un-infested leaves and infested leaves at 7 and 17 days of aphid infestation in four Fabaceae species (*Pisums ativum* L., *Vicia faba* L., *Trifolium pretense* L, *Medicago sativ*a L.). Feeding by pea aphids *Acyrthosiphon pisum* Harris caused significant loss of chlorophyll *a* and *b* in the infested plants. Un-infested leaves on both short- and long-infestation plants had significantly higher chlorophyll *a* and *b* than infested leaves.

Layla and Al-Shareef (2011) studied effect of the sweet potato whitefly, *Bemisia tabaci* (Gennadius), infestation on the mean content of plant pigments (chlorophyll *a*, chlorophyll *b* and carotene), as well as the percentage of moisture content in the leaves of three different plant varieties (cantaloupe, cucumber and zucchini) in greenhouse. Results indicated that plant pigments (chlorophyll *a*, chlorophyll *b* and carotene)

differed significantly in their contents in the plant varieties. In general, mean content of chlorophyll *a* was significantly higher than chlorophyll *b*, which was higher than carotene. Plant varieties differed significantly in their content of pigments. Infestation with *B. tabaci* reduced mean content of each plant pigment (chlorophyll *a*, chlorophyll *b* and carotene) in all plant varieties. Feeding of the whitefly decreased total percentage of moisture content in all plant varieties as well.

Mahadeva (2011) studied the changes in biochemical constituents (free amino acids, total soluble proteins, total reducing sugars, total soluble sugars, starch and total phenols) and photosynthetic pigments (total chlorophyll, chlorophyll *a*, chlorophyll *b*, chlorophyll *a/b* ratio and carotenoids) in thrips (*Pseudodendrothrips mori* Niwa) infested mulberry foliage of six popular varieties (M5, MR2, Mysore local, S36, S54 and V1). It was found that there was a significant variation in biochemical constituents and photosynthetic pigments in the thrips infested mulberry leaves compared to healthy leaves. The post-infestational changes in these components lead to imbalance in nutritional status of mulberry leaves which adversely influenced the growth and development of silkworm as well as quality of silk produced.

Prabhakar *et al.* (2012) conducted field studies with eight blackgram genotypes having differential response to YMD (Yellow Mosaic Disease). Comparison of mean reflectance spectra of the healthy and YMD infested leaves showed changes in all the broad band regions. However, reflectance sensitivity analysis of the narrow-band hyperspectral data revealed a sharp increase in reflectance from the diseased leaves compared to healthy at 669 (red), 505 and 510 nm (blue). ANOVA showed a significant decrease in leaf chlorophyll with increase in disease severity, while no such relationship was observed for relative water content. By plotting coefficients of determination (R^2) between leaf chlorophyll and per cent reflectance at one nm wavelength interval, two individual bands (R571; R705) and two band ratios (R571/R721; R705/R593) with highest R^2 values were selected. These bands showed a significant linear relationship with SPAD chlorophyll readings (R^2 range 0.781-0.814) and spectrometric estimates of total chlorophyll content (R^2 range 0.477-0.565).

Simpson *et al*. (2012) studied the potential interaction of water stress on plant physiology of cabbage and on development of *Myzus persicae* Sulz. Plants under combined aphid and water stress showed significantly reduced SPAD and final biomass, with comparatively higher leaf water potentials. It was deduced that aphid infestation prevents solute accumulation in the vacuole of a drought stressed cabbage and may be an attempt to increase local turgor or prevent too great a difference in osmotic potential between themselves and the host. At the same time changes in the host, akin to drought stress takes place.

Hubert *et al*. (2013) measured the total chlorophyll (Chl *a*+*b*) content in seedling leaves of fifteen maize cultivars infested by two aphid species (*R. padi* and *Sitobion avenae* F.), 7 and 14 days after the infestation, using a SPAD-502 chlorophyll meter . Chlorophyll loss was more severe in *R. padi*-infested than in *S. avenae*-infested plants. Chlorophyll depletion was greater after long-term (14 days) than after short-term (7days) aphid infestation.

Luczak *et al*. (2013) studied on the content of green (chlorophyll *a*+*b*) and yellow-orange (carotenoids) pigments in the leaves of 7 cultivars of spinach *viz.*, Olbrzym Zimowy, Orbita, Greta, Rembrandt F1, Markiza, Matador, Spiros F1 and New Zealand spinach and their effect on black bean aphid (*Aphis fabae* Scop.). The largest numbers of total aphids and colonies were observed on the cultivar Olbrzym Zimowy, whereas, the fewest ones on New Zealand spinach. The high percentages of plants infested by total aphids and winged migrants, and the largest numbers of winged aphids were found on two cultivars: Matador and Markiza. The cultivar Matador was characterized by the highest yellow-orange pigment content in leaves and the highest ratio of these pigments to green ones. On the cultivar Spiros F1, small numbers of aphids and small percentages of infested plants were observed. Also, it was characterized by the lowest content of pigments (chlorophyll *a*+*b*, carotenoids) in leaves. The influence of yellow-orange pigments on the *A. fabae* invasion was confirmed by the statistically significant and positive correlation coefficients between the carotenoids to chlorophyll (*a*+*b*) ratio and the number of winged aphids and colonies, and also the percentages of infested plants by total aphids and winged migrants.

Agnieszka (2014) studied the effect of feeding by the grain aphid *S. avenae* on chlorophyll, carotenoid and flavonoid content in waxy and waxless triticale genotypes. On both sampling dates (5 and 10 days after infestation), seedlings of infested waxy and waxless plants had lower chlorophylls and carotenoids and higher flavonoids than in uninfested plants.

Haresh and Shashank (2014) observed that the chlorophyll and carotenoid contents of uninfested and infested leaves of guava by scale insect, *Aonidiella orientalis* (Newstead) were reduced by 31 and 35%, respectively by using 118-spectrophotometer.

Golan *et al*. (2015) determined the plant responses to *Coccus hesperidum* L. infestation on two host plants. Groups of five lemon and five fern plants were colonized by various numbers of mobile *C. hesperidum* instar nymphs. After 6 months, all scale insect individuals were counted on each plant. According to the insect density, the plants were divided into a five-degree series. In all density classes of host plants tested, the infestation of scale insects decreased the chlorophyll and carotenoid content as well as the value of three indicators of photosynthetic activity. The strongest decrease in the analyzed pigments was observed for the smallest abundance of insects (first class density, 10 individuals per leaf) in lemon leaves and in second-class density (11 to 30 individuals per leaf) in fern leaves. The strongest reactions of the chlorophyll fluorescence indicators were observed in density classes III (from 31 to 50 individuals per leaf) and IV (from 51 to 100 individuals per leaf) in the fern leaves and density classes IV or V (more than 100 individuals per leaf) in the lemon leaves. The reactions depended on the specific properties of plants and abundance of insects feeding on them.

Tagger *et al.* (2015) studied the effect of feeding by whitefly, *B. tabaci* on chlorophyll loss as quantified from the leaves of nine black gram genotypes over a period of two seasons. The chlorophyll content indices declined significantly in all the genotypes under whitefly-infested conditions, both at 30 and 50 days after sowing (DAS) particularly in the lower canopy of the plants. During both seasons, the moderately resistant genotypes (NDU 5-7 and KU 99-20) recorded lowest per cent decrease in the chlorophyll content, whereas highly susceptible genotypes (KU 7-504 and KU 7-505) recorded highest per cent decrease in the chlorophyll contents under whitefly-infested conditions.

2.3 EFFECT OF INSECT FEEDING ON RELATIVE WATER CONTENT (RWC)

Sanjay *et al.* (1984) showed variations in both biochemical and biological parameters when *Epilachna dodecastigma* (Wied) was cultured on water stressed and unstressed leaves of bitter gourd. Proline, free amino acid and steroid content increased while total protein, carbohydrate, ascorbic acid and relative water content decreased in the water stressed leaves. The larval and pupal duration increased in insects cultured with stressed leaves. The increased duration and decreased body weight of *E. dodecastigma* could presumably be due to higher accumulation of sesquiterpenoid like compounds or poor food quality in stressed leaves or low food water intake by insects grown on stressed leaves.

Walter (1989) studied on influence of Russian wheat aphid infestation on the response of barley plants to drought stress. Fourteen-day-old plants were infested with eight apterous adult aphids, which were removed 7 days later with systemic insecticide. Leaves previously infested with aphids had lower relative water content, reduced stomatal conductance, more negative water potential, lower levels of chlorophyll and higher levels of amino-N, proline and glycine-betaine than corresponding leaves from un-infested plants. When water was withheld for a period of 7 days after aphids were removed, the relative water content of previously infested plants dropped steadily from 0.89 to 0.60, while the relative water content of un-infested plants remained at about 0.94 for the first 4 days of the drought stress period followed by a steady drop to about 0.77 by the end of the drought stress period. Leaf water potentials dropped steadily during the drought stress period in both previously infested (-1.14 to -1.91 mPa) and un-infested (-0.54 to -1.52 mPa) plants. It was concluded that Russian wheat aphids cause drought-stress symptoms in leaves of infested plants even in the presence of ample root moisture. The observations of low levels of glycine-betaine and proline present in leaves after water was withheld from roots and lack of leaf growth upon alleviation of drought stress in previously-infested plants, suggested that aphid infestation limits the capacity of barley plants to adjust successfully to drought stress.

Cabera *et al.* (1995) compared the effects of aphid infestation with some effects of wounding and drought-stress, several physiological parameters and metabolite

concentrations in infested, mechanically wounded or water-stressed young barley plants. Barley plants infested with the greenbug, *S. graminum* had lower water potentials and CO_2 assimilation than non-infested plants. Abscisic acid content increased by 55% in leaves after 72 h of infestation. Water potentials and stomatal resistance of barley plants changed only as a consequence of infestation by the greenbug or by drought-stress. These results showed that greenbug infestation of barley produced changes similar to those observed in plants subjected to drought-stress and that aphids feeding on both groups of seedlings had lower developmental and mean relative growth rates. Water-stress caused in barley by aphid infestation or drought would probably affect greenbug development due to the effects of stress on the chemical composition of the plant.

Schmidt *et al.* (2009) reported that after short term *Spodoptera littoralis* Boisduval feeding, leaf growth and water content decreased in damaged leaves. The glutamate/glutamine ratio increased and other free amino acids were also affected. In contrast, mild spider mite (*Tetranychus urticae* Koch) infestation did not affect leaf growth or amino acid composition, but led to an increase in total nitrogen and sucrose concentrations. Both herbivores induced locally increased dark respiration, suggesting an increased mobilization of storage compounds potentially available for synthesis of defensive substances, but did not affect assimilation and transpiration. Systemically induced leaves were not significantly affected by the treatments. The results showed that cotton plants do not compensate the loss of photosynthetic tissue with higher photosynthetic efficiency of the remaining tissue. However, early plant responses to different herbivores leave their signature in primary metabolism, affecting leaf growth. Changes in amino acid concentrations, total nitrogen and sucrose content may affect subsequent herbivore performance.

Prabhakar *et al*. (2011) conducted studies to characterise leaf hopper (LH) stress on cotton, identify sensitive bands, and derive hyper spectral vegetation indices specific to this pest. Cotton plants with varying levels of LH severity were selected from three locations across major cotton growing regions of India. Reflectance from healthy and leafhopper infested plants showed a significant difference in VIS and NIR regions. Decrease in chl *a* pigment was more significant than chl *b* in the infested plants and the

ratio of chl *a*/*b* showed a decreasing trend with increase in LH severity. Regression analysis revealed a significant linear relation between LH severity and chl (R^2= 0.505), and a similar fit was also observed for RWC (R^2= 0.402).

Prabhakar *et al*. (2013) conducted a study to characterize reflectance spectra of cotton plants with known mealybug infestation levels (grade-0: healthy and grade-4: severe), and seek to identify specific narrow wavelengths sensitive to mealybug damage. Significant differences were found in green, near infrared and short wave infrared spectral regions for plants with early stages of *Phenacoccus solenopsis* Tinsley infestation, and for plants showing higher grades of infestation these differences extended to all the regions except blue. A significant reduction in total chlorophyll (12.83–35.83%) and relative water content (1.93–23.49%) were observed in the infested plants.

A series of no-choice experiments were conducted to investigate multiple plant responses in six Brassica crops to feeding by *Bagrada hilaris* Burmeister (Huang *et al*, 2014). Varying numbers of adults were caged onto cotyledon, 2-true leaf, and 4-true leaf-stage plants of broccoli, green cabbage, red cabbage, cauliflower, kale, and radish for a 48 h infestation period. Feeding damage on leaf surfaces, total leaf area, and relative chlorophyll content on plants of each crop were measured before and after the 48 h infestation period. In addition, dry weights and total leaf area for the 4-leaf-stage plants were measured at 21 days post-infestation to estimate the residual impacts on older plants. In all crops tested, feeding damage increased with greater numbers of *B. hilaris* adults caged on cotyledon and 2-leaf-stage plants. Significant reductions in leaf area, relative chlorophyll content, and dry weight in all crops indicated negative impacts on plant growth by *B. hilaris*. Moreover, cotyledon and 2-leaf plants were more severely impacted by *B. hilaris* induced injury than the 4-leaf plants, and kale appeared to be less sensitive to *B. hilaris* feeding than the other five Brassicaceous hosts.

Rani *et al.* (2014) studied the abiotic stress induced changes in physiological, biochemical and oxidative level reactions caused by drought stress in betel and castor plants and their influence on the feeding performance of herbivore, *Spodoptera litura* Fabricius. Under drought stress, the leaf chlorophyll and relative water content (RWC) in both the test plants decreased than controls. The decrease in the individual phenolic

acids in both plants due to stress caused by water deficit was determined using HPLC analysis. The reduced levels of primary metabolites were evident in both plants, while flavonoid content enhanced along with amino acid content in castor plants. These changes created a favorable environment for *S. litura* and increased the feeding rate in both drought affected castor and betel plants than their normal plants. Increased levels of anti-oxidative enzymes [superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT)] in leaves of betel and castor plants indicated their defensive and/or protective role against the ROS (Reactive oxygen species) generated under drought conditions. It was concluded that drought conditions induced the defense responses in both betel and castor plants, which regulated the herbivore feeding performance.

CHAPTER III

MATERIAL AND METHODS

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Studies on biology of corn leaf aphid, *R. maidis* and relationship between insect density, leaf pigments and relative water content (RWC) in sorghum due to aphid feeding were carried out in the Entomology laboratory and green house, CRIDA, Santhoshnagar, Hyderabad with the following objectives.

1. To study the biology of the corn leaf aphid, *R. maidis* on sorghum
2. To establish relationship between insect density and leaf pigments
3. To estimate the relative water content (RWC) in sorghum due to aphid feeding.

3.1 BIOLOGY OF THE CORN LEAF APHID *R. maidis* ON SORGHUM

Cohort was taken from fields of CRIDA and Hayathnagar Research Farm. For studying the biology of aphids, Petri plates (9 cm dia.) with filter paper disc lining the bottom were taken and sprinkled with water. Fresh sorghum leaves were provided as feed. The cut end of sorghum leaf was wrapped with wet cotton wool at one edge to keep the leaf fresh and turgid for a longer period of time. The 1st instar nymph was transferred @ one nymph/plate to the fresh leaf with the help of fine camel hair brush for its development (Plate 1). These Petri plates were kept in Environmental Test Chamber (SANYO Versatile Environmental Test Chamber) at temperature of 27±0.5^{o}C, light 4 LS and RH 65±5%. Every day readings were taken and at every two days leaves were changed with fresh one. The 100 individuals morphometrics (length and breadth in µm) were measured by using stereo zoom microscope (OLYMPUS SZX10) (Progress caption 2.7). The change of instars was identified based on the presence of exuviae cast by the nymph. The number of instars, total duration of life span and the number of newly emerged nymphs were noted (Plate 2 A-F).

Plate 1. Studies on biology: Release of first instar nymph

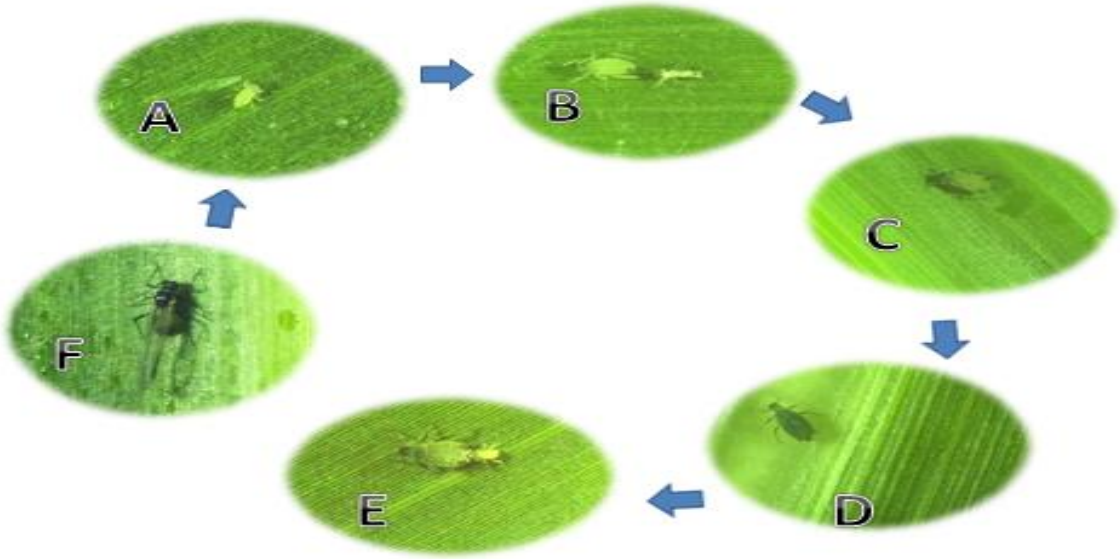


Plate 2. Biology of *R. maidis* A. First instar B. Second instar C. Third instar D. Fourth instar E. Adult laying nymph F. Winged adult

3.1.1 Calculation of life-table statistics

The data on mortality was recorded daily till all the adults died during rearing of aphids in Petri plates. The method suggested by Morris and Miller (1954) was used for constructing life-tables. From 100 nymphs, the number of alive and dead were recorded daily and the following column heads were used in the documentation of age-specific life table.

x : Age of the insect

l_x : Number of individuals that survive at the beginning of each age interval 'x' out of 100

d_x : Number of individuals that died during the age interval 'x' out of 100

$100\ q_x$: Per cent mortality

$100\ q_x = (d_x/l_x) \times 100$

The survivorship curve was drawn by plotting the number of living individuals at a given age (l_x) and mortality (d_x) against the age (x). The shape of the curve described the distribution of mortality with age (Slobodkin, 1962).

3.1.2 Construction of life and fertility tables of *R. maidis* on sorghum

The number of nymphs laid by the female on each day was counted till the death of the adults. The life table for female was constructed from column l_x as described by Birch (1948) and Poole (1974).

x : Pivotal age of female in days

l_x : Number of females alive at the beginning of each age interval 'x' (as fraction of initial population of one)

m_x : Average number of nymphs laid per female in each age interval assuming 1 as sex ratio

3.1.3 Estimation of population growth attributes

3.1.3.1 Net reproductive rate (R_0): The values of 'x', 'l_x' and 'm_x' were calculated from the data given in life tables. The sum total of the products '$l_x \times m_x$' is the net reproductive rate (R_0) (Lotka, 1925). The 'R_0' is the rate of multiplication of population in generation measured in terms of females produced per generation. The number of

times a population would multiply per generation was calculated by the following formula,

$$R_0 = \sum lx \; x \; mx$$

3.1.3.2 Mean generation time (T_c): The appropriate value of generation time (T_c) *i.e.*, the mean age of the mothers in a cohort at the birth of female offspring was calculated by using the following formula.

$$T_c = \frac{\sum lx \; x \; mx \; x \; x}{R_0}$$

3.1.3.3 Innate capacity for increase (r_c):

$$r_c = \frac{\log_e R_0}{T}$$

Where, e = Natural log (*i.e.*, 2.71828)

The above r_c is an approximate value of intrinsic rate of natural increase (r_m) and is slightly lower than r_m value for insects with overlapping generations as suggested by Laughin (1965) and Southwood (1978).

3.1.3.4 Intrinsic rate of increase (r_m): The approximate value of r_c and other provisional values r_m were substituted in the following equation to obtain accurate value of intrinsic rate of increase r_m.

$$\sum e^{-r.mx} \; x \; lxmx = 1$$

3.1.3.5 Corrected generation time (T): It was calculated by using the following formula

$$T = \frac{\log_e R_0}{r_m}$$

3.2 ESTIMATION OF LOSSES OF LEAF PIGMENTS DUE TO *R. maidis* (Fitch)

3.2.1 Maintenance of corn leaf aphid culture

Sorghum was raised in plastic pots (10 × 10 cm dia.) in greenhouse. The aphids collected from CRIDA field were released on 30 days old potted sorghum plants @ 100-200 aphids/plant. When yellowing/chlorosis of the top leaves was noticed, fresh, uninfested 30 days old potted sorghum plants were offered. Later, these aphids served as a source of population for conducting various physiological experiments.

3.2.2 Maintenance of sorghum plants in pots

Sorghum SPV-462 seedlings were raised in pots (10 × 10 cm dia.) in green house. The pots were filled with one kg soil and four seeds/pot were dibbled and watered. When the seedlings were 3, 13 and 23 days old, water soluble fertilizer POORNA-19 was applied @ 0.5 g/plant (Plate 3 A-B). The five day old seedlings were isolated with mylar tubes to prevent the infestation of insect pests (Plate 4).

3.2.3 Release of aphids on plants

The leaves of 25 days old potted seedlings of sorghum cultivar (SPV-462) were infested with a mixed population of nymphs and adult aphids @ 100, 200 and 300 individuals per plant and graded as given below. Uninfested seedlings were kept as control (Plate 5 A-C).

Table 3.1 Grading of sorghum plants infested by aphids

No. of nymphs and adult aphids released/plant	Infestation
100	Low
200	Medium
300	High
Nil	Control

Plate 3. A. Raising of sorghum seedlings in pots B. Exclusion of insect pests by covering with mylar tube

Plate 4. Aphid population on sorghum plant

A. Chlorosis of leaves

B. Presence of exuviae

C. Rolled leaves

Plate 5. Effects of aphid feeding on sorghum plants

3.2.4 Physiological parameters

Observations were recorded on the progression of damage to leaves at 20th day after release of different densities of aphids. Physiological parameters *viz.*, chlorophyll content, fluorescence and photosynthetic rate were measured using SPAD chlorophyll meter, Chlorophyll fluorometer and IR Gas Analyser (Plate 6 A, B and C), respectively. Also, the loss in leaf pigments *viz.*, chlorophyll (*a*+*b*) and carotenoid were quantified as per the methods suggested by Porra *et al.* (1989), Wang *et al*. (2004) and Haresh and Shashank (2014) and flavonoids by Kreft *et al.* (2002).

3.2.4.1 Chlorophyll content: The SPAD 502 meter (Soil Plant Analytical Development) (Minolta, Japan) is a simple hand held and portable instrument which provides information on the relative amount of leaf chlorophyll. Before measurement, the instrument was calibrated and light transmission was measured with no leaf inside. Then a leaf was clamped by the meter which absorbs a certain portion of red light. The meter calculates a relative value (in SPAD units), showing how green the leaf is. The SPAD chlorophyll index data was measured (n = 29) by averaging three readings from the index leaf *i.e.,* third leaf from the top at 20 days after release of the aphids.

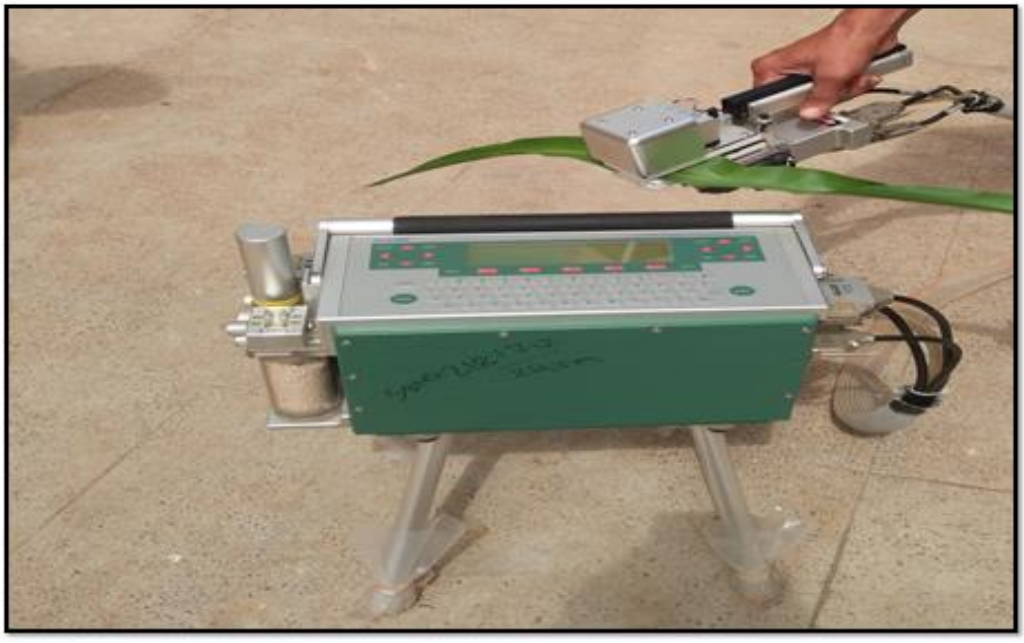
3.2.4.2 Chlorophyll fluorescence ratio (Fv/Fm): The measurement of chlorophyll fluorescence is both non-destructive and non-invasive, and thus has considerable potential for use in the field situation. Applications range simply from a means of rapidly identifying injury to leaves in the absence of visible symptoms to a detailed analysis of causes of change in photosynthetic capacity.

Chlorophyll fluorescence was measured at 20 days after release of the aphids on plant leaf whorl. Chlorophyll fluorescence characteristics were measured on damaged leaves using a fluorometer (Model FluorPen FP 100) and used to estimate the extent of damage induced photo inhibition. The measured data was sequentially stored in the internal FluorPen memory. Comprehensive FluorPen 1.0 software provides data transfer routines and many additional features for data presentation in tables and graphs.

The Fv/Fm ratio parameters were determined following the procedures of Maxwell and Johnson (2000) and used to quantify the degree of damage of whorl leaves induced photo inhibition.

A. SPAD chlorophyll meter

B. Chlorophyll fluorometer



C. Infra Red Gas Analyser

Plate 6. Recording of physiological parameters in sorghum plant

3.2.4.3 Photosynthetic rates: Infra-Red Gas Analyser measures the reduction in transmission of infra-red wavebands caused by the presence of a gas between the radiation source and a detector. It consists of various parts like leaf chamber, air flow meter and means of generating and controlling air flow over the leaf, heating or cooling a small portion of the air system. Leaf chambers have humidity sensor for measuring transpiration and light sensor for measuring irradiance (Mulkey and Smith, 2007).

By using portable photosynthesis system *i.e.,* Infra-Red Gas Analyser (Model LI-6400, LI-COR, USA) instantaneous measurement of the photosynthesis rates (µmol CO_2 m^{-2} sec^{-1}) were obtained from different densities of aphid infestation.

3.2.4.4 Leaf Chlorophyll concentration: The third leaf of sorghum from top was cut after taking the SPAD readings. 500 mg of the leaf was weighed and placed in vial with 25 ml dimethyl sulphoxide (DMSO) for chlorophyll extraction. The sample vials (three replications) were incubated at room temperature in the dark for 48 h to allow for complete extraction of chlorophyll into the solution. Absorbance of the clear extracts was measured using Genesys UV/VIS spectrophotometer (Thermospectronic, Rochester, USA) at 663 and 645 nm (Plate 7 A-B). The amount of Chl *a*, *b* and Chl (*a*+*b*) were computed from the absorption coefficients (Porra *et al.,* 1989). Concentration of leaf chlorophyll was expressed as mg/g.

Chl *a* = (12.7 x 663nm-2.69 x 645nm) x V/1000 x FW

Chl *b* = (22.9 x 645nm-4.68 x 663nm) x V/1000 x FW

Chl (*a*+*b*) = (20.2 x 645nm+8.02 x 663nm) x V/1000 x FW

Where,

V= volume of the DMSO

FW = Fresh weight of the leaf sample

3.2.4.5 Leaf carotenoid concentration: 100 mg of the third leaf was weighed and placed in a vial with 25 ml dimethyl sulphoxide (DMSO) for carotene extraction. The sample vials (three replications) were incubated at room temperature in the dark for 48 h to allow for complete extraction of chlorophyll into the solution. Absorbance of the clear extracts was measured using Genesys UV/VIS spectrophotometer

A. Leaf extract

B. Spectrophotometer

Plate 7. Extraction of leaf pigments for analysis in spectrophotometer

(Thermospectronic, Rochester, USA) at 480 nm. Concentration of leaf carotenoid as mg/g was calculated by using the following formula (Hiscox and Israelstam, 1979)

$$\text{Carotenoid} = \frac{[(1000 \text{ x } A_{480\text{nm}}) - 1.29 \text{ x Chl } a - 53.78 \text{ x chl } b]}{420}$$

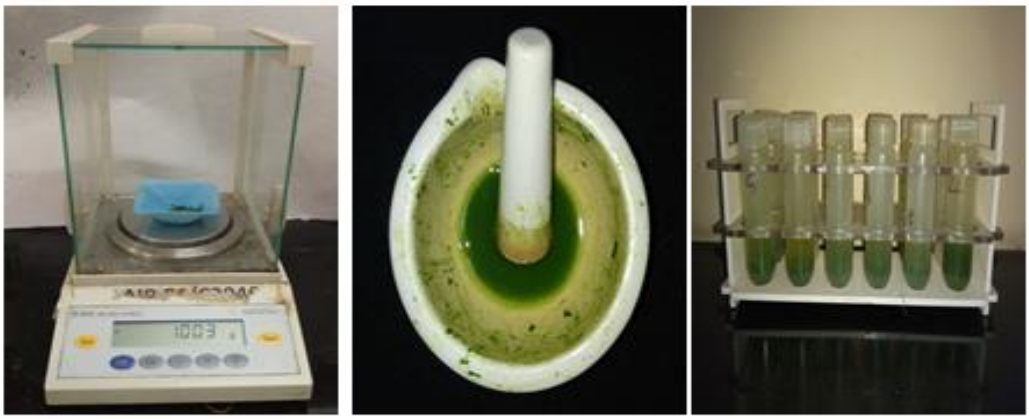
3.2.4.6 Leaf flavonoid content: One gram of the third leaf was weighed and added to a mixture of acetone: methanol: water at 1:1:1 ratio (3.33ml each). The leaf was crushed by using mortar and pestle, the extract was centrifuged at 1000 rpm for 10 min and filtered by using filter paper. From the filtrate, 2 ml of extract solution was mixed with 2 ml of 2% Aluminum chloride ($AlCl_3$) in methanol. The mixture was incubated for 10 min at room temperature, and the absorbance was measured at 420 nm in Genesys UV/VIS spectrophotometer (Thermospectronic, Rochester, USA) against blank samples (Plate 8 A-F). The total concentration of flavonoid in the extract was determined as microgram of Rutin Equivalent (RE) according to the formula that was obtained from standard rutin graph.

Absorbance = 0.0144 x Total Flavonoid (µg Rutin Equivalent) + 0.0556

3.3 ESTIMATION OF RELATIVE WATER CONTENT (RWC) IN SORGHUM DUE TO APHID FEEDING

500 mg of leaf sample (3rd leaf from top) was weighed for RWC analysis. After recording the fresh weight (FW), the leaves were cut into small pieces and were soaked in water for 4 h for recording turgid weight (TW). Thereafter, the samples were dried at 80^{o}C for about 48 h to determine the dry weight (DW) (Plate 9 A-B). RWC was calculated as described by Smart and Bingham (1976).

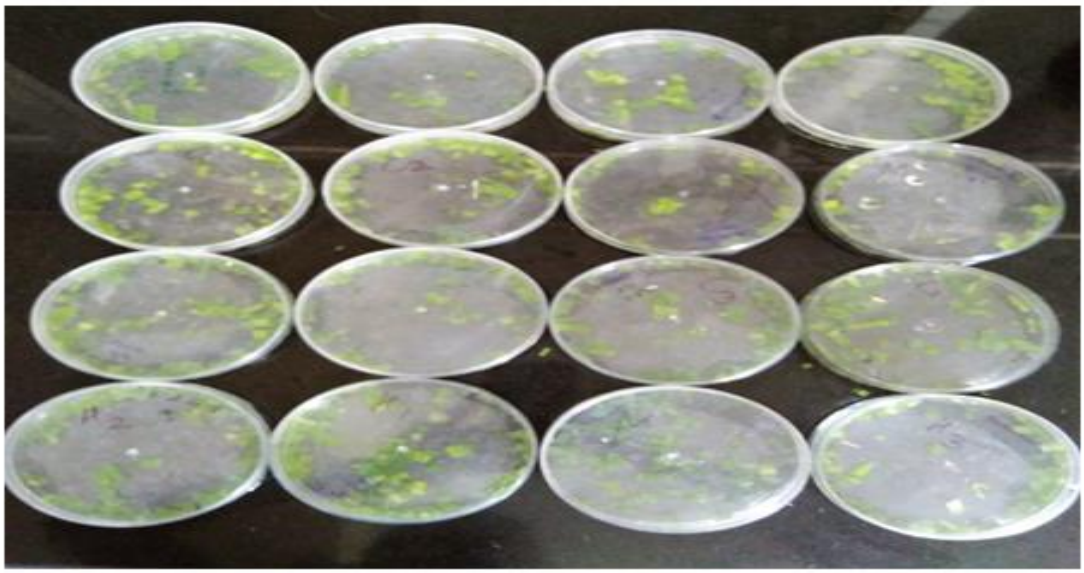
$$\text{RWC} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}}$$



A. Weighing of leaf sample B. Crushed leaves C. Collected leaf extract

D. Centrifuge E. Filtering F. 2% $AlCl_3$ solution

Plate 8. Procedure for flavonoid content extraction



A. Soaking of pieces of sorghum leaves in water

B. Determination of leaves dry weight

Plate 9. Estimation of relative water content (RWC)

3.4 STATISTICAL ANALYSIS

Life table analysis and graphs were generated using computer software package MS EXECL (windows 10). Significant effects of aphid damage on leaf pigments concentration (chl *a*, chl *b*, chl (*a*+*b*), carotenoid and flavonoid), SPAD chlorophyll content, fluorescence, photosynthesis rate and RWC were determined by analysis of variance (ANOVA). Simple correlation coefficients were used to evaluate relationship of plant physiological parameters with the different densities of pest released in three levels of infestation (grade 1-3). The software package SPSS (Superior Performance Software System, USA, version 16.0) was used for performing statistical analysis.

CHAPTER IV

RESULTS AND DISCUSSION

Chapter IV

RESULTS AND DISCUSSION

The results obtained were presented under the following headings.

4.1 Biology of the corn leaf aphid, *R. maidis* on sorghum

4.2 Relationship between insect density and leaf pigments

4.3 Estimation of relative water content (RWC) in sorghum due to aphid feeding

4.1 BIOLOGY OF THE CORN LEAF APHID, *R. maidis* ON SORGHUM

Corn leaf aphid was found to pass through four nymphal instars before becoming adults. The apterous females reproduced parthenogenetically and were found to directly give birth to nymphs. All the nymphs produced developed into females. No males were encountered in the present studies. The duration of various biological parameters and morphological data of different stages are given in table 1, fig. 1 and table 2, fig. 2, respectively.

4.1.1 First instar nymph

The first instar nymph was light green in colour. The tips of head, antennae and legs were slightly darker than the body. It was very active and found to move fast on the surface of leaf. The duration of first instar on an average was 1.97±0.02 days at 27^{o}C. The morphometrics were recorded by using microscope and the length and width were found to be 720.57±9.95 µm and 303.62 ±5.59 µm, respectively.

4.1.2 Second instar nymph

The second instar nymph was found to be less active and pale green in color. The head, abdomen and antennae were darker than the body and the legs were paler. The total duration of second instar on an average was 2±0.03 days. Body length was 978.46±13.51 µm and width was 412.83±5.53 µm.

4.1.3 Third instar nymph

The third instar body was still pale green, but slightly darker on the sides. Legs were darker than the body. The head was dark green in color. On an average it took 2.02±0.04 days to complete this instar. Body length was 1399.52±24.56 µm and width was 581.74±12.33 µm.

4.1.4 Fourth instar nymph

The fourth instar took 1.98±0.03 days to become adult. Body length was 1737.5±20.28 µm and width 733.98 ±10.96 µm.

4.1.5 Adults

The total life cycle from nymphal stage to adult stage was found to be 7.79±0.12 days. The adults were either winged (alate) or wingless (apterous). Apterae were rather elongate aphids with short antennae. The colour was yellowish green to dark olive green or bluish green and measured 1833.63±15.89 µm in length and 783.26±10.82 µm in width. The two cornicles (projections arising from the top rear of the abdomen) were dark, relatively short, and surrounded by a dark basal area. The winged forms had two pairs of delicate transparent wings. The adult longevity was 12.27±0.27 days. The life span of *R. maidis* on sorghum was found to be 16.25±0.80 days. The fecundity rate was 35.97 progenies/female and the rate of reproduction was 9.15 progenies/female/day.

The study of biology is important for understanding the form and extent of its population growth. It is difficult to study the life history traits, including development, longevity, fecundity and population growth statistics of *R. maidis* under field condition due to interference of biotic and abiotic factors. Hence, studies under laboratory condition assumes significance in understanding the population growth and there by formulating management tactics.

In the present studies, two distinct phenologies were found in aphid cycle, *i.e.,* nymphs and adults. The development from nymphs to adult took 7.97±0.03 days, with the nymphs undergoing four moults to become females. The mean duration of first, second, third, and fourth instar of *R. maidis* was 1.99±0.02, 2±0.03, 2.02±0.04 and 1.98±0.03 days, respectively at 27^{o}C. However, El-Sheikh *et al.* (2009) reported that the total life cycle of *R. maidis* on barley was 5.0±1.6 days at 25^{o}C with the developing

period of various instars being 1.4±0.5, 1.2±0.4, 1.1±0.2 and 1.4±0.5 days, respectively. Similarly, Mei *et al.* (2006) also reported that the life cycle of *R. maidis* was 5.7±0.2 days at 25^{o}C on corn with the developmental rate of first, second, third, and fourth instar being 1.5±0.1, 1.5±0.1, 1.4±0.1 and 1.4±0.1 days, respectively. Thus, the duration of developmental time of various nymphal instars in the present study showed slight variation from earlier studies which could be due to difference in temperature and the host plant on which the insects were reared. Earlier Mei *et al*. (2006) reported that 30^{o}C was most optimal temperature for immature development among the five temperature tested *viz.*, 6, 10, 15, 20, 25, 30 and 35^{o}C. While, El-Sheikh *et al.* (2009) reported that the developmental period of nymphal stage decreased with increase in temperature up to 25^{o}C.

The adult longevity and life span of *R. maidis* in the present study was found to be 12.27±0.27 and 16.25±0.80 days on sorghum which was similar to that reported by Mei *et al*. (2006) and El-Sheikh *et al*. (2009), the values being 12.0±0.9 and 17.7±1.1 days on corn and 11.9±3.4 and 16.9±3.2 days on barley, respectively. Thus, adult longevity and total life span were almost similar at 27^{o}C and 25^{o}C though the hosts were different. Auad *et al.* (2009) reported that the life cycle, adult longevity and life span of *R. padi* was 7.13±0.11, 10.00±0.40 and 19.97±1.83 days on signal grass at 24^{o}C, 7.22±0.4, 8.4±0.8 and 15.6±0.8 days for *A. forbesi* on strawberry at 25±2^{o}C (Araujo *et al*., 2015) 8.3±0.83, 25.6±4.60 and 33.9±4.58 days at ambient condition, respectively for *B. brassicae* on cabbage (Meenakshi *et al.*, 2014). The aphid biological parameters varied with the species, type of the host plant offered and the geographic origin of the aphid indicating that these factors can affect its development even when kept under similar heat condition (Smith, 1922).

The rate of reproduction was found to be 9.15 nymphs per day and average fecundity rate was 35.97 progenies/female at 27^{o}C. Chaudhary *et al*. (1968) observed that the rate of reproduction and fecundity were 2.1 nymphs/female/day and 34.2 progenies/female at 19^{o}C on wheat. Other researchers *viz.*, Foott (1977), Silva Maia (2004), Mei *et al*. (2006) and El-Sheikh *et al*. (2009) reported that average fecundity rates were 68.06, 69.45, 34.2 and 28.31 progenies/female respectively at 25^{o}C and they opined that with increase in temperature the fecundity reduced. The rate of reproduction

and fecundity in the other species *viz.*, *R. padi*, *A. forbesi*, *Hyadaphis foeniculi* (Passerini) and *C. lanigera* were found to be 2.73±0.14 nymphs per day and 19.97±1.83 progenies/female (Auad *et al.,* 2009), 1.5±0.3 nymphs per day and 8.2±1.7 progenies/female (Araujo *et al*., 2015), 1.20±0.09 nymphs per day and 13.50±2.25 progenies/female (Ramalho *et al.,* 2015) and 3 to 5 nymphs per day and 41 to 56.6 progenies/female (Joshi and Viraktamath 2014), respectively. Adams (2007) reported that nutrional factors are also responsible for variation in fecundity, in which an increase in nitrogen content was responsible for higher fecundity of *R. padi.*

From our findings, it can be concluded that *R. maidis* on sorghum had a life cycle of 7.79±0.12 days, adult longevity of 12.27±0.27 days, life span of 16.25±0.80 days while the fecundity and rate of reproduction were 35.97 progenies/female and 9.15 progenies/female/day, respectively.

The length and width of different stages of *R. maidis* were: 720.57± 9.95 µm and 303.62±5.59 µm (1^{st} instar); 978.46 ±13.51 µm and 412.83±5.53 µm (2^{nd} instar); 1399.52±24.56 µm and 581.74±12.33 µm (3^{rd} instar) and 1737.5±20.28 µm and 733.98±10.96 µm (4^{th} instar), respectively (Table 4.2 and Fig. 2). Patil and Patel (2013) and Aheibam *et al.* (2015) reported the length of different stages of *A. gossypii* as 0.510±0.007 and 0.51±0.06 mm (1^{st} instar); 0.790±0.014 and 0.70±0.09 mm (2^{nd} instar); 1.140±0.012 and 0.95±0.06 mm (3^{rd} instar) and 1.390±0.013 and 1.16±0.09 mm (4^{th} instar), respectively, while the corresponding width was 0.38±0.005 and 0.25±0.03 mm (1^{st} instar); 0.47±0.010 and 0.38±0.03 mm (2^{nd} instar); 0.59±0.006 and 0.53±0.07 mm (3^{rd} instar) and 0.71±0.008 and 0.67±0.02 mm (4^{th} instar).

The size of *R. maidis* adult was 1833.63±15.89 µm in length and 783.26±10.82 µm in width. Veronica *et al.* (1997) reported that the adult body length of *S. graminum* was 1.87±0.261 mm. It is almost similar to *R. maidis.* Hayder and Nassreen (2012) reported the adult length to be 1.60-2.30, 1.65-2.25 and 1.32-1.75 mm for *R. maidis*, *R. padi* and *S. graminum* respectively. Dixon *et al.* (1982) reported that these variations were due to influence of temperature and food quality.

Table 4.1. Biological parameters of *R. maidis* on sorghum

Parameters	Values Obtained*
1st instar (days)	1.97±0.02
2nd instar (days)	2.00±0.03
3rd instar (days)	2.02±0.04
4th instar (days)	1.98±0.03
Total life cycle (days)	7.79±0.12
Adult longevity (days)	12.27±0.27
Life span (days)	16.25±0.80
Fecundity rate (progeny/female)	35.97
Rate of reproduction (progeny/female/day)	9.15
N (Number of insects)	100

***(Mean ± SEM)**

Table 4.2. Morphometrics of *R. maidis* on sorghum

Life stages	Morphological parameters*	
	Length (µm)	**Width (µm)**
1st instar	720.57± 9.95	303.62 ± 5.59
2nd instar	978.46 ± 13.51	412.83 ± 5.53
3rd instar	1399.52 ± 24.56	581.74 ± 12.33
4th instar	1737.5 ± 20.28	733.98 ± 10.96
Adult Female	1833.63 ± 15.89	783.26 ± 10.82

***(Mean ± SEM)**

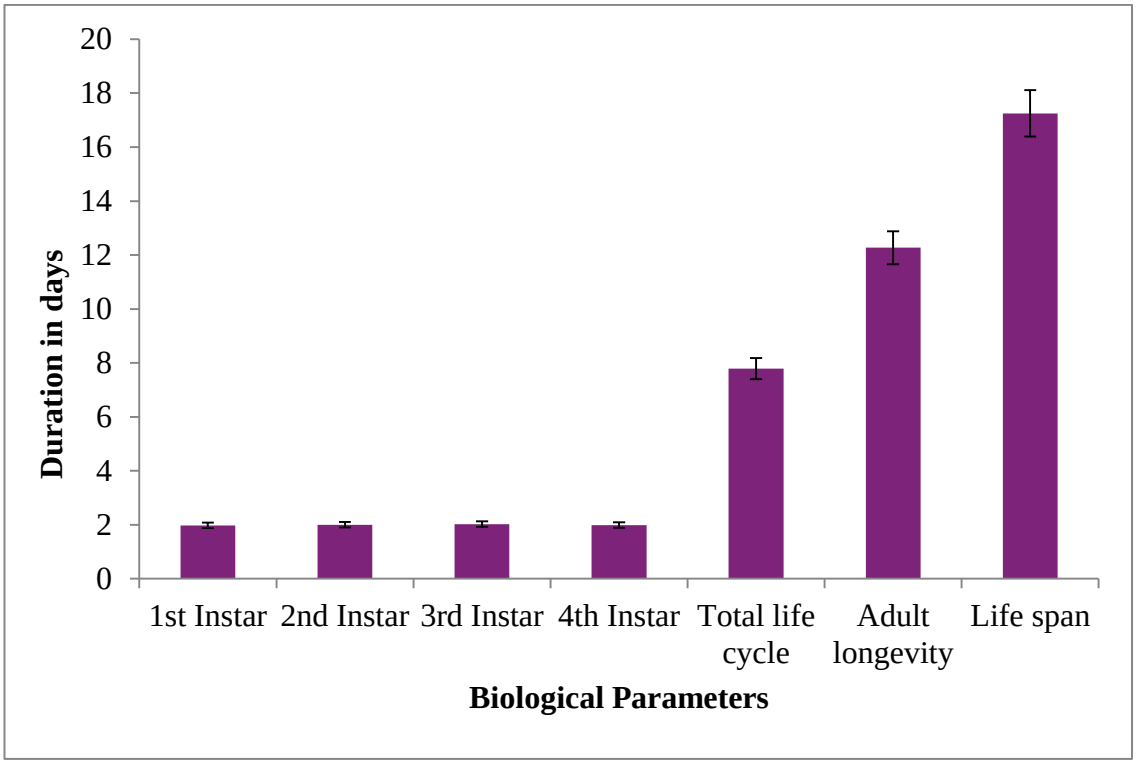


Fig. 1. Duration of various parameters of *R. maidis* on sorghum leaves

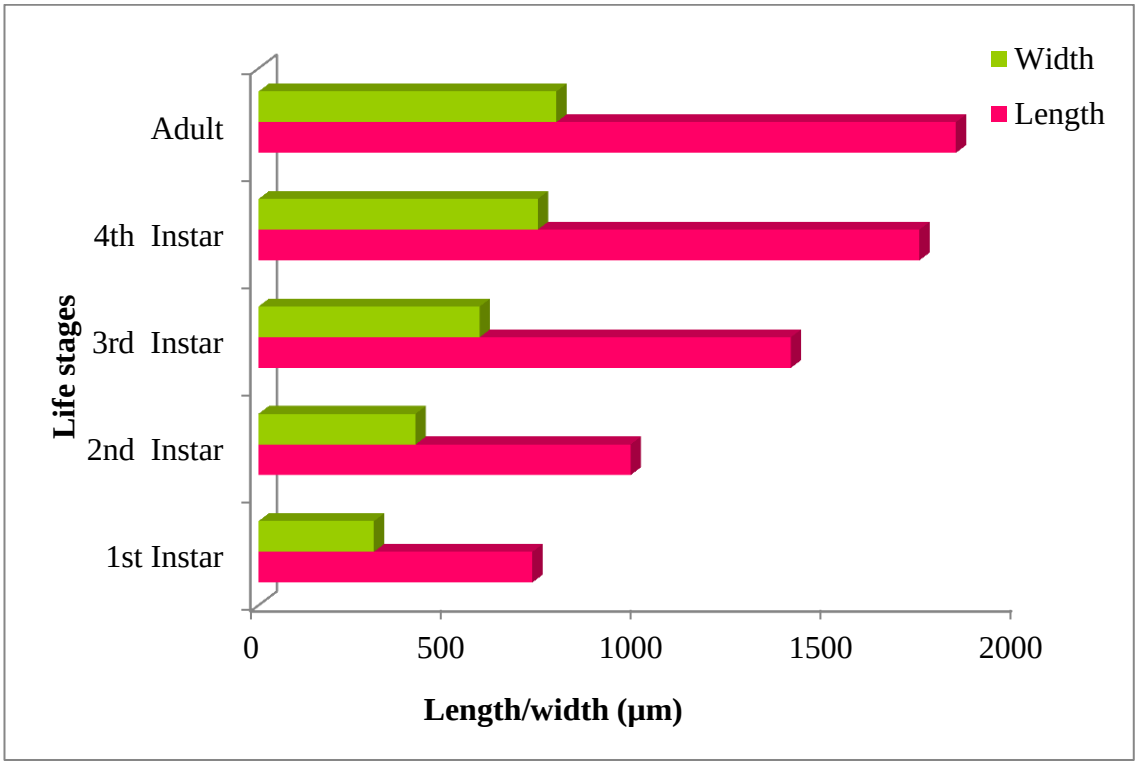


Fig. 2. Morphometrics of *R. maidis* on sorghum

4.1.6 The age-specific survivorship

The age-specific life-table of the *R. maidis* was worked out by observing the survivals (l_x) and mortality (d_x) during the specific age (x). The results are given in table 4.3.

The survival rate (l_x) showed lower mortality during early growth. The survivals remained constant for first three days (100 each). However, from fourth day the survivals of the population showed a steady decline (Fig. 3). The survivals of the population between 9^{th} to 11^{th} day remained relatively constant due to lower mortality during early adult stage. The mortality was more pronounced after 11^{th} day. The adult mortality was observed during 12^{th} to 16^{th} day. Out of 100 nymphs, 72 nymphs moulted to adults successfully on 7^{th} day. The population took 16 days to complete the life span at 27^{o}C on sorghum leaf.

The deleterious effect of high temperatures on survivorship is very common in various aphids (Morgan *et al*., 2001 and Wang and Tsai, 2001). The age-specific survivorship (l_x) curves decreased more rapidly and even more sharply as the duration of different life stages increased from 4 to 16 days (Fig. 3). At 27^{o}C, 50% mortality occurred on the 15^{th} day, and all aphids died on the 16^{th} day. According to Mei *et al*. (2006) at 35^{o}C, 50% of mortality occurred on the 7^{th} day, and all aphids died on the 14^{th} day and also confirmed that, for higher temperatures, the age-specific survival curves dropped more sharply and quickly when temperatures increased from 20 to 35^{o}C.

4.1.7 The life and fertility table

Life and fertility table provides the information about the longevity, fecundity, age specific survivals of adult female. In the present study, the life and fecundity table of *R. maidis* was calculated on sorghum leaves and the results obtained are given in table 4.4 and 4.5.

The 1^{st} instar nymph took about 7 days to become adult and immediately it started producing nymphs. The number of progenies /female/day (m_x) was >10 up to 11^{th} day and declined in the later days of the life span (Fig. 4). The female contributed to the highest number of progenies (m_x = 21.77) on the 9^{th} day of pivotal age (x). The fecundity peak started when females were three days old. The female *R. maidis*

Table 4.3. Age-specific life table of *R. maidis* on sorghum

Age of the insects in days (x)	No. of individuals surviving at the beginning of each age interval x out of 100 (l_x)	No. of individuals dying during the age interval x out of 100 (d_x)	Per cent mortality rate at the age interval x (100 q_x)
1	100	0.00	0.00
2	100	0.00	0.00
3	100	5.00	5.00
4	95	9.00	5.26
5	90	9.00	10.00
6	81	12.00	11.11
7	72	12.00	16.66
8	60	1.00	20.00
9	48	1.00	2.08
10	47	12.00	2.13
11	46	7.00	26.08
12	34	7.00	20.58
13	27	7.00	25.93
14	20	4.00	20.00
15	16	8.00	50.00
16	8	8.00	100.00

Table 4.4. Life and fertility table of *R. maidis* on sorghum

Pivotal age of female in days (x)	No. of individuals surviving at the beginning of each age interval x out of 100 (l_x)	No. of progenies/female/day (m_x)
1	100	-
2	100	-
3	100	-
4	95	-
5	90	-
6	81	-
7	72	10.07
8	60	14.75
9	48	21.77
10	47	18.28
11	46	14.64
12	34	4.52
13	27	3.63
14	20	2.10
15	16	1.25
16	8	0.50

production was 10.07 to 14.75 nymphs in a 24 h period on sorghum while, Hesler *et al.* (2005) 7.5 to 11.4 nymphs by *R. padi* on transgenic wheat plants. The effect of the host plant on nymph production was also reported by Hesler (2005) where in the *R. padi*, during 7^{th} day of pivotal age (x) produced between 23.6 and 43.3 nymphs/female.

The reproduction rate (R_0) was 45.05 at 27^{o}C (Table 4.5). Jabraeil Razmjou and Ali Golizadeh (2013) reported that R_0 value was 33.50 on wheat cultivar Tajan at 25^{o}C. Earlier Abd El-Rahman (1997) reported R_0 value of 35.09 for *S. graminum* at 24^{o}C, although Mei *et al*. (2006) and El-Sheikh *et al*. (2009) reported the value to be 33.1 and 21.5 respectively, at 25^{o}C.

The intrinsic rate of increase (r_m) relates to (R_0) and corrected mean generation time (T), indicating the biotic potential of the species (Traicevski and Ward, 2002). According to Mei *et al*. (2006) values of r_m, which reflect the overall effects of temperature on development, reproduction, and survival of a population, increased as temperature increased from 6 to 25^{o}C, consistent with the trend of the developmental rate. The optimum temperature for the highest population growth potential of the corn leaf aphid occurred at around 25^{o}C and the maximum r_m was 0.329. At 27^{o}C, the value of r_m in our study was 0.44 (Table 4.5) while Mei *et al*. (2006) and El-Sheikh *et al*. (2009) reported the values of r_m as 0.32 at 25^{o}C and almost similar value (0.27 and 0.28) at 20^{o}C on different hosts. Asin and Pons (2001) reported that r_m was 0.30 at 25^{o}C for *S. avenae* and 0.24 at 22^{o}C for *Metopolophium dirhodum* (Walker), but it is much lower than the 0.52 at 27.5^{o}C for *R. padi.* Hence, the results indicate that corn leaf aphids probably are better adapted in population growth to a wider range of high temperatures in warm regions, similar to that reported in a previous study (El-Ibrashy *et al*., 1972).

The time interval between each generation (T) *i.e.,* 8.73 days was recorded for *R. maidis* at 27^{o}C (Table 4.5). El-sheikh *et al.* (2009) reported the T value as 9.52 days at 25^{o}C for the same aphid. While it was 9.62 and 11.45 days for *R. padi* and *S. graminum* on barley, respectively (El-Heneidy *et al.,* 2004), and was 9.43 days for *R. padi* on Signal grass at 24^{o}C (Auad *et al.,* 2009). Adams and Drew (1964) opined that corn leaf aphids have short developmental times to maturation and high lifetime fecundity on some grasses at 21.1^{o}C similar to that on sorghum in the present study and on barely (Elliott *et al.,* 1988). This conforms with the report that widely distributed grasses are

Table 4.5. Life table parameters of *R. maidis* on sorghum

Parameters	Values
Net reproduction rate (R_0)	45.05
Mean generation time (T_c)	9.24
Intrinsic rate of natural increase (r_m)	0.44
Innate capacity of increase (r_c)	0.41
Corrected mean generation time (T) in days	8.73

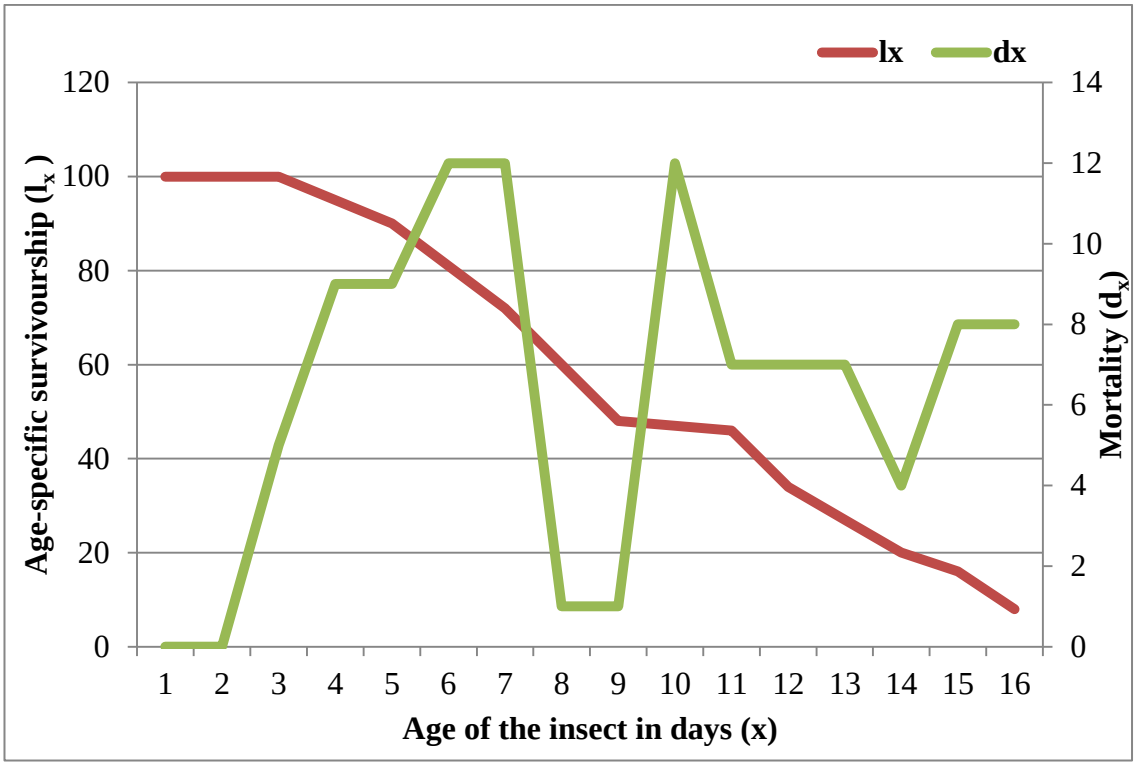


Fig. 3. Age-specific survivorship (l_x) and mortality (d_x) of *R. maidis*

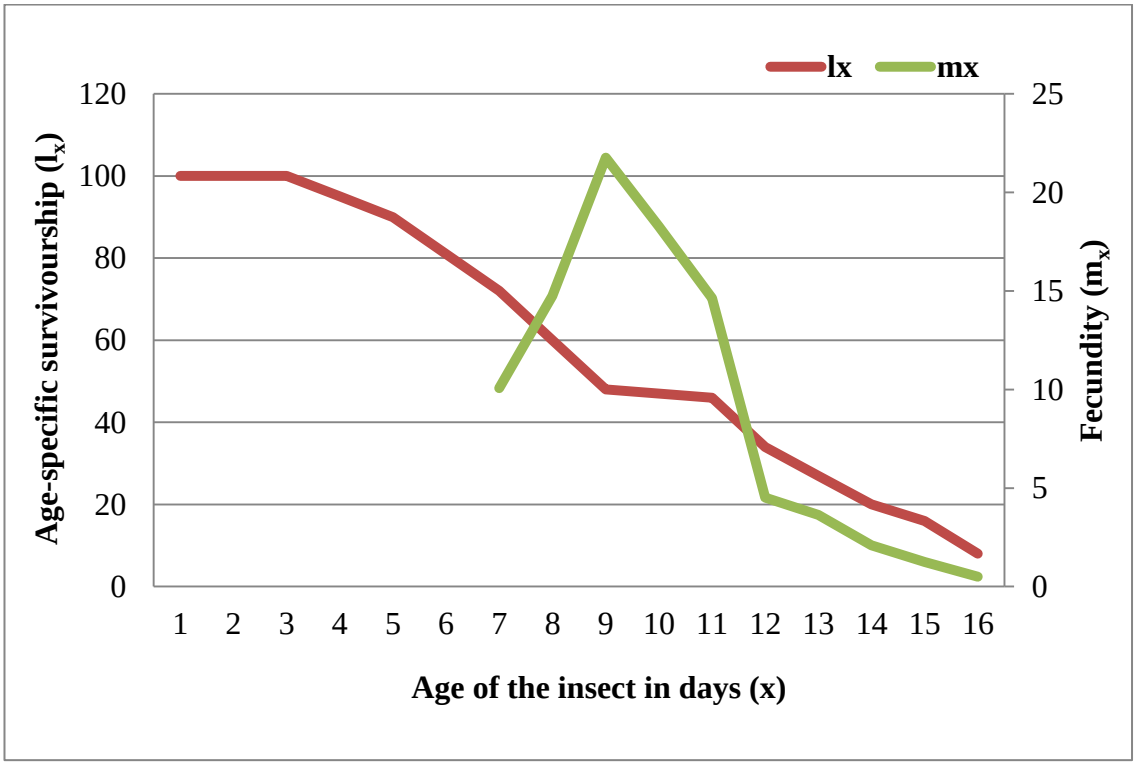


Fig. 4. Age-specific survivorship (l_x) and fecundity (m_x) of *R. maidis*

good alternative host plants for the maintenance of aphid populations (Weibull, 1993 and Perng, 2002).

In the present study, the net reproduction rate (R_0) of corn leaf aphid was 45.05, mean generation time (T_c) 9.24, intrinsic rate of natural increase (r_m) 0.44, innate capacity of increase (r_c) 0.41 and corrected mean generation time (T) 8.73 days on sorghum.

Thus the study of biology of aphid and life table parameters provided information regarding longer life span of adults and thereby higher food requirements leading to the visibility of the pest and symptoms, respectively, on the sorghum crop and can thus be utilized for proper assessment for use of control measures in the field. Hence, this information will be helpful during the development of successful Integrated Pest Management Programme (IPM) for *R. maidis*.

4.2 RELATIONSHIP BETWEEN INSECT DENSITY AND LEAF PIGMENTS

4.2.1 Effect of aphid infestation on chlorophyll content in SPAD units

SPAD (Soil and Plant Analyzer Development) values obtained from each plant were averaged for further analyses. Average values of SPAD readings decreased under the stress of *R. maidis* feeding. The values under high, medium and low infestation (27.1±0.74, 33.4±0.39 and 35.1±0.44, respectively) showed significant difference over no infestation (38.6±0.60) (Table 4.6 and Fig. 5). The differences in leaf chlorophyll content (SPAD units) between the different infestation levels is highly significant ($p < 0.0001$). *R. maidis* infestation on sorghum leaves showed 9.06-29.79% decline in chlorophyll content (SPAD units) over no infestation. According to Smith *et al*. (2005), *D. noxia* stressed leaves of wheat cv. Wichita showed ~53% decline in total chl content (SPAD units) versus control leaves; isogenic lines PI 372129 and P243781 showed chlorophyll loss of 53% and 21%, respectively.

Nagaraj *et al*. (2005) used the SPAD technique to assess feeding damage by greenbug, *S. graminum* feeding on sorghum. Feeding by *B. tabaci* was reported to reduce relative leaf chlorophyll levels (SPAD values) in lettuce leaves (Palumbo *et al*, 1996). Many workers have reported on the destructive effects of aphid infestation on the

functioning of various mono and dicotyledonous plant systems (Sempruch *et al*., 2011; Chen *et al*., 2012; Bak *et al*., 2013), one such effect being chlorosis (Botha *et al*., 2006 and Golawska *et al*., 2010).

In the present data, the chlorophyll content (SPAD units) in un-infested sorghum plants was significantly higher than in aphid-infested plants. A significant advantage of the use of SPAD instrument is that it can quantify chlorophyll non destructively in plant tissues in situ.

4.2.2 Effect of aphid infestation on chlorophyll fluorescence (Fv/Fm)

The relative level of the maximum quantum efficiency of photosystem PSII (Fv/Fm) in the leaves of sorghum due to *R. maidis* infestation was characterized by a decrease compared to the control (Table 4.6 and Fig. 6). The lowest level of this parameter was noted in high infestation (0.69±0.01) and it showed significant difference over no infestation (0.74±0.01), while the medium (0.72±0.01) and low infestation (0.73±0.01) were on par with no infestation.

The feeding of leaf corn aphids on sorghum leaves caused a decrease in the Fv/Fm level to a minimum value of 1.35% (low infestation) to a maximum of 6.75% (high infestation) compared to the control. The results of the ANOVA test in the experiment was F = 9.06, df = 3, 16 and P = 0.001 for Fv/Fm.

Fv/Fm value indicate the efficiency of the photochemical system *i.e.,* specifically how much light energy captured is being used by the reaction center and propagated through the photoelectron transport chain. Measuring Fv/Fm provides a rapid method for determining changes in the maximum efficiency of PSII photochemistry (Andrews *et al.,* 1995). The relative decrease in Fv/Fm has also been used in the rapid assessment of plant susceptibility or resistance to aphids (Blanco *et al.,* 1992). This parameter is widely considered to be a sensitive indication of plant photosynthetic performance. Its lower values would be observed with some types of biotic or abiotic stress factors, which reduce the capacity for photochemical quenching of energy within PSII (Kalaji and Guo, 2008). An effective quantum yield of the PSII of light-adapted leaves is a good indicator of the efficiency of light utilisation, *i.e.,* how

efficiently absorbed photons are converted into chemical products (Malkin and Niyogi, 2000).

In this experiment, Fv/Fm was significantly affected by *R. maidis* infestation in sorghum plants. It is typical of the response of many plants to a wide range of environmental stresses and indicates a reduced efficiency of PSII photochemistry (Krause and Weis, 1991; Chaerle *et al.*, 2007; Nabity *et al.*, 2009). There was a substantial decrease in chlorophyll fluorescence parameter in sorghum leaves with high aphid infestation (300 aphids/plant). According to Dai *et al.* (2009) the primary mechanism for photosynthetic rate reduction in damaged leaves is via the interference of the photochemical efficiency at the initial stage of photosynthesis. Velikova *et al.* (2010) reported a significant reduction in the chlorophyll fluorescence parameters of herbaceous plants resulting from the feeding and oviposition of stinkbug, *Murgantia histrionica* (Hahn) and also confirmed that the permanent impairment of photosynthetic photochemistry was restricted to the damaged areas on the leaf.

4.2.3 Effect of aphid infestation on photosynthetic rate

The significant reduction in photosynthetic rate was seen in infested plants compared to uninfested plants. The photosynthetic rate (µmol CO_2 m^{-2} sec^{-1}) in sorghum plants with high infestation was 13.2±1.23 which was significantly different from no infestation (19.5±1.02) (Table 4.6 and Fig. 7). However, there was no much variation in photosynthetic rates of low (18.3±0.6) and medium infested plants (17.6 ±0.65).

Nagaraj *et al*. (2002) found that greenbug, *S. graminum* feeding affected photosynthetic rate more than it affected chlorophyll content in sorghum. Under infestation by feeding stinkbugs *M. histrionica*, photosynthesis decreased rapidly and substantially in cabbage and common bean but in savoy cabbage leaves emission of mono and sesquiterpenes was induced (Velikova *et al.*, 2010). Also, Haile *et al*. (1999) and Heng-Moss *et al*. (2003) opined that the significant decline of the photosynthetic rate in aphid-injured leaves might have resulted from increased synthesis of chemical defense compounds in response to herbivory.

In our study 6.15, 9.74 and 32.30% reduction in photosynthetic rate was noticed at low, medium and high infestation levels respectively, compared to no infestation

Table 4.6. Effect of different levels of aphid infestation on physiological parameters in sorghum

Attributes	Chlorophyll content* (SPAD units)	% reduction in Chlorophyll content	Fluorescence* (Fv/Fm)	% reduction in Fluorescence	Photosynthetic rate* ($\mu molCO_2m^{-2}sec^{-1}$)	% reduction in Photosynthetic rate
No Infestation (control)	38.6 ± 0.60^{a}	-	0.74 ± 0.00^{a}	-	19.5 ± 1.02^{a}	-
Low Infestation	35.1 ± 0.44^{b}	9.06	0.73 ± 0.01^{a}	1.35	18.3 ± 0.6^{a}	6.15
Medium Infestation	33.4 ± 0.39^{b}	13.47	0.72 ± 0.01^{a}	2.70	17.6 ± 0.65^{a}	9.74
High Infestation	27.1 ± 0.74^{c}	29.79	0.69 ± 0.01^{b}	6.75	13.2 ± 1.23^{b}	32.30
df	3,25	-	3,24	-	3,16	-
F value	72.10	-	10.71	-	9.06	-
P value	<.0001	-	0.0001	-	0.001	-

Means within a column followed by the same letter are not significantly different using Tukey's HSD test (α=0.05); df: degrees of freedom.

***(Mean±SEM)**

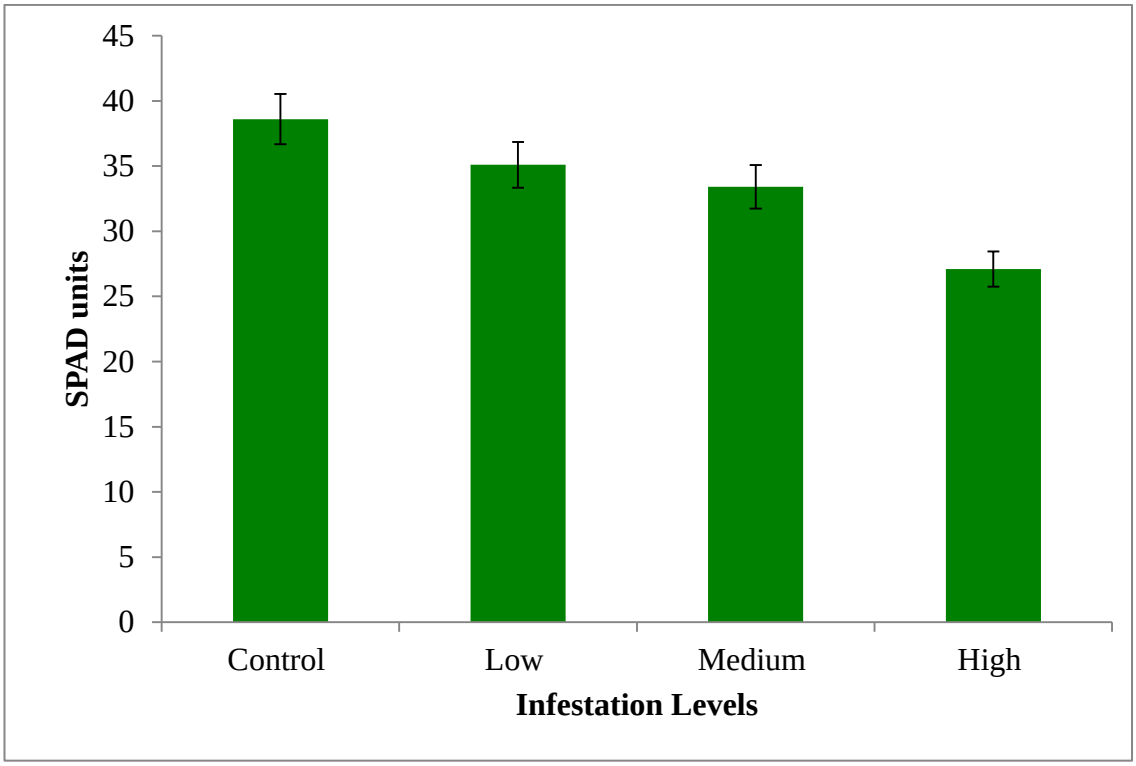


Fig. 5. Effect of different levels of aphid infestation on chlorophyll content in sorghum

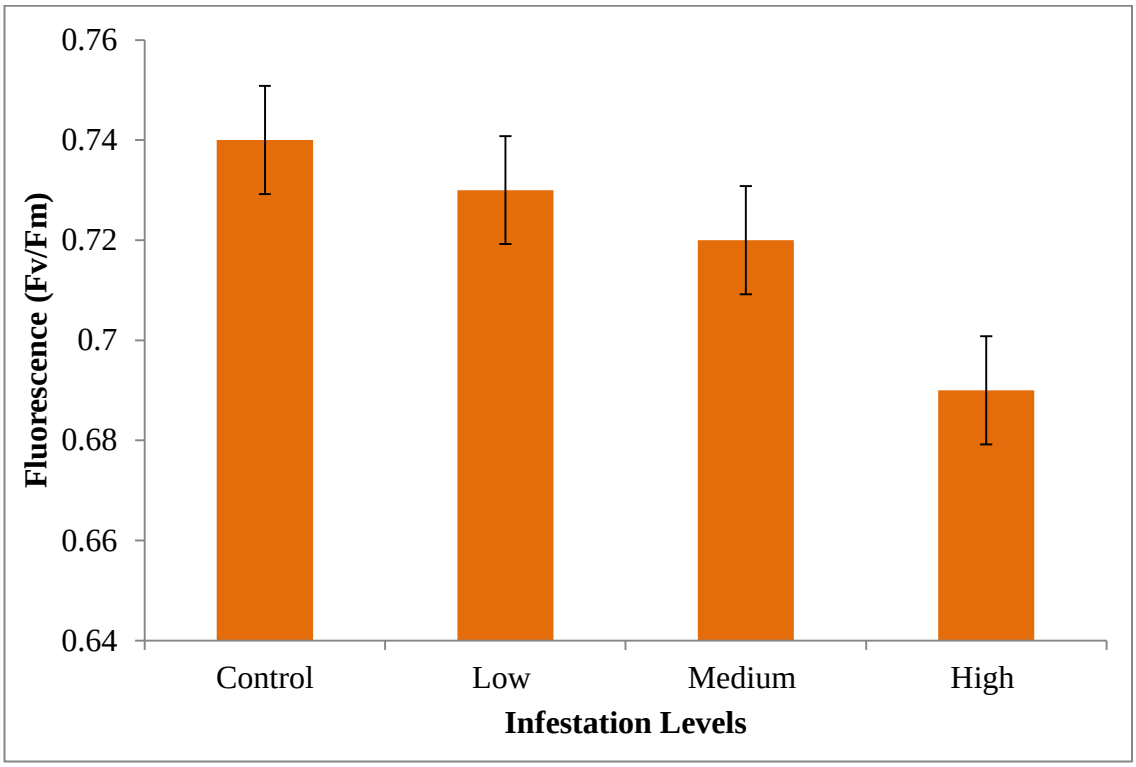


Fig. 6. Effect of different levels of aphid infestation on chlorophyll fluorescence in sorghum

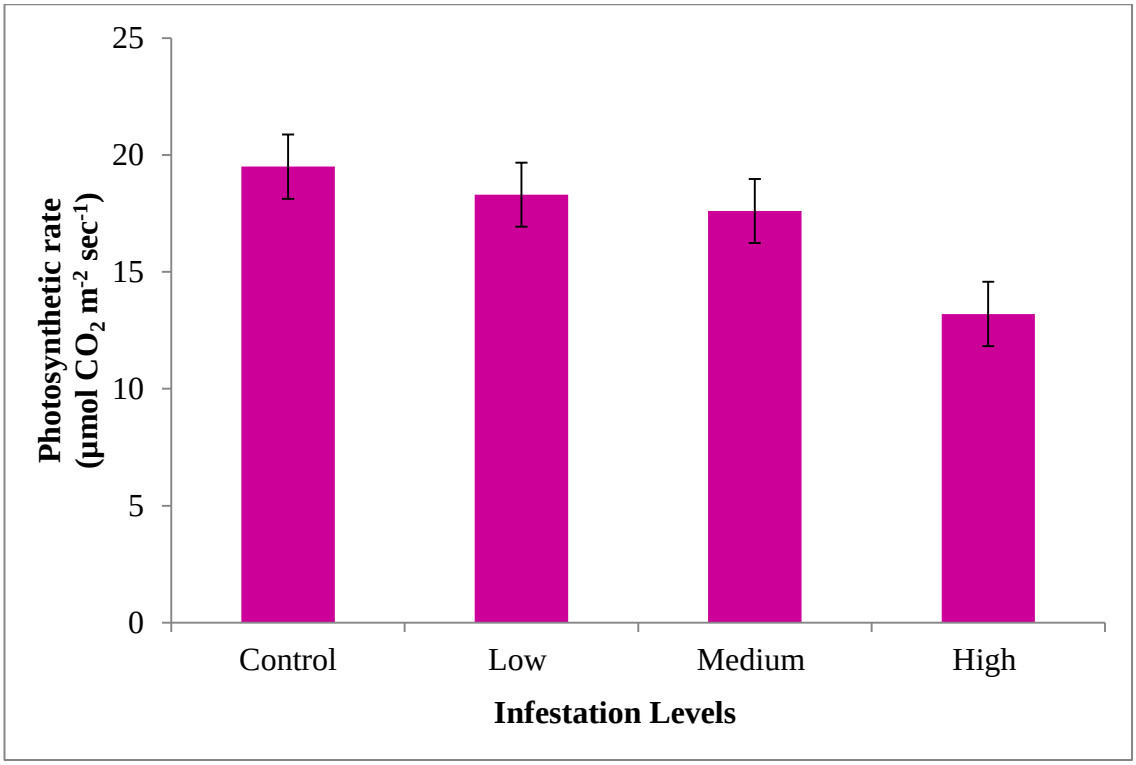


Fig. 7. Effect of different levels of aphid infestation on photosynthetic rate in sorghum

(Table 4.6). According to Macedo *et al*. (2003) soybean aphids, *A. glycines*, even in low densities (20 aphids/leaflet) were responsible for photosynthetic rate reductions of as much as 50% on infested soybean even though leaflets with no apparent symptoms of aphid injury such as chlorosis were noticed.

4.2.4 Correlation studies between insect density and physiological parameters in sorghum

Correlation coefficient between insect density with physiological parameters *viz.*, SPAD chlorophyll content, chlorophyll fluorescence and photosynthetic rate are given in table 4.7 and fig. 8.

The chlorophyll fluorescence and photosynthetic rate revealed a negative but non-significant correlation at low infestation. Significant negative correlation was obtained between low insect density and SPAD chlorophyll content (r = -0.48019) which indicated decrease in the SPAD chlorophyll content with increase in insect density.

At medium infestation, negative non-significant correlation was observed between insect density and fluorescence but significant negative correlation was obtained with SPAD chlorophyll content (r = -0.54562) and photosynthetic rate (r = -0.50763) which indicated that an increase in insect density decreased chlorophyll content and photosynthetic rate.

A negative and significant correlation was observed between insect density and all the three parameters *viz.*, chlorophyll content (r = -0.80396) fluorescence (r = -0.30266) and photosynthesis rate (r = -0.80316) at high infestation.

The results of the present study on the infestation level of aphids was similar with the results of Golan *et al*. (2015) who reported that infestation of scale insect at different density classes (first, second, third, fourth and fifth class density-10, 11 to 30, 31 to 50, 51 to 100 and more than 100 individuals per leaf, respectively) decreased the chlorophyll and carotenoid content and chlorophyll fluorescence on lemon and fern plants. Ademir *et al*. (2006) reported a negative correlation between infestation level and photosynthesis on Rangpur lime and coffee leaves, which were infested with scale and coffee leaf miner, respectively. Buntin *et al*. (1996) showed that feeding injury

Table 4.7. Correlation matrix of insect density with physiological parameters in sorghum

Attributes	Insect density	Chlorophyll content	Fluorescence (Fv/Fm)	Photosynthetic rate (µmol $CO_2m^{-2}sec^{-1}$)
Low infestation				
Insect density	1.000	-	-	-
Chlorophyll	-0.48019*****	1.000	-	-
Fluorescence	-0.17302	0.246338	1.000	-
Photosynthetic rate	-0.42509	0.548966	-0.12741	1.000
Medium infestation				
Insect density	1.000	-	-	-
Chlorophyll	-0.54562*****	1.000	-	-
Fluorescence	-0.27339	0.221935	1.000	-
Photosynthetic rate	-0.50763*****	-0.89439	-0.3604	1.000
High infestation				
Insect density	1.000	-	-	-
Chlorophyll	-0.80396*****	1.000	-	-
Fluorescence	-0.30266*****	-0.06996	1.000	-
Photosynthetic rate	-0.80316*****	0.837021	0.145736	1.000

*****Significant at 5% level

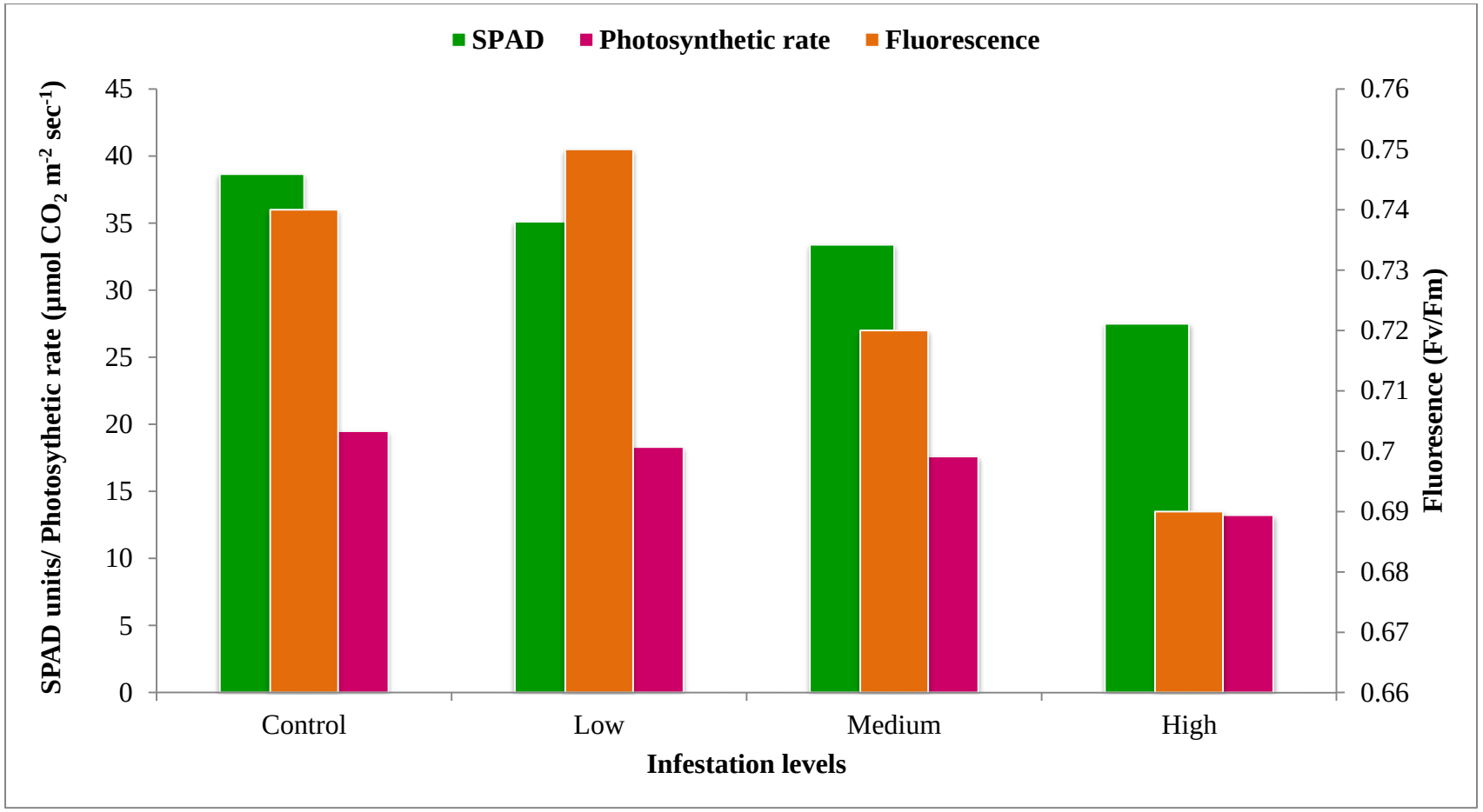


Fig. 8. Effect of different levels of aphid infestation on physiological parameters in sorghum

caused by *Stephanitis pyrioides* (Scott) reduced the chlorophyll content and adversely affected net leaf photosynthesis and transpiration in azalea. The feeding of *B. tabac*i reduced the leaf photosynthesis in tomato leaves by decreasing the content and photosynthetic capacity of chlorophyll (Buntin *et al*., 1993). John *et al.* (2007) reported that reduction in chlorophyll content affected the photosynthetic capacity of susceptible soybean when *A. glycines* population increased through time.

From the present studies, it is clearly evident that low infestation (100 aphids/plant) affected chlorophyll content, medium infestation (200 aphids/plant) affected chlorophyll and photosynthetic rate while, high infestation (300 aphids/plant) affected all the three parameters significantly.

4.2.5 Effect of aphid infestation on concentration of leaf pigments

Chemical analyses showed that aphid infestation altered the levels of the studied pigments. The mean values of chlorophyll and carotenoid concentration are given in table 4.8.

4.2.5.1 Chlorophyll *a* concentration

Spectrophotometric estimation of chlorophyll from healthy and aphid infested plants showed a significant decrease in chl *a* with increase in number of aphids (Fig. 9). It was found that low infestation of *R. maidis* on sorghum leaves caused over 17.37% and 39.76% (high infestation) decrease in chl *a* content compared to no infestation. The medium infestation (16.6±1.19 mg/g) showed highly significant difference over no infestation (25.9±1.41 mg/g). The results of the ANOVA test were as follows for chl *a* content: F = 5.64, df = 3, 25 and P = 0.0043.

4.2.5.2 Chlorophyll *b* concentration

In the leaves of sorghum infested with *R. maidis*, a decrease in tendency of chl *b* level compared to control plants was observed (Fig. 10). The lowest content of the chl *b* (1.5±0.17 mg/g) was found in leaves infested with 200 aphids (medium infestation) and it showed significant difference (2.8±0.23 mg/g) over no infestation but was on par with low (2.4±0.34 mg/g) and high (1.9±0.41 mg/g) infestation. The results of the ANOVA test were as follows for chl *b* content: F = 3.15, df = 3, 25 and P = 0.00426.

4.2.5.3 Chlorophyll *a*+*b* concentration

R. maidis infestation caused a significant loss in chl (*a*+*b*) concentration in sorghum leaves. The chl (*a*+*b*) content in medium (18.1±1.30 mg/g) and high infestation (17.5±3.09 mg/g) showed significant difference over no infestation (28.7±1.63 mg/g). However, the chl (*a*+*b*) in low infestation (23.8±2.25 mg/g) was on par with no infestation. The decrease in chl (*a*+*b*) content was significant (P = 0.0054) at medium and high infestation levels.

4.2.5.4 Carotenoid content

The leaves of sorghum infested with *R. maidis* showed a tendency in decrease of carotenoid level compared to control. The lowest level of the analysed parameter was noted in high infestation *i.e*., 0.6±0.09 mg/g of the leaf (Fig. 11). The aphid infested leaves showed significant (F= 6.25, df = 3, 25 and P = 0.0026) differences in carotenoid content between the infested (medium and high) and un-infested leaves.

From the results obtained it is evident that the chl (*a*+*b*) and carotenoid content decreased significantly in medium (200 aphids/plant) and high infestation (300 aphids/plant) compared to low infestation (100 aphids/plant). It was also noticed that the leaves in the upper canopy of the plants harbour greater number of nymphs and adults which continuously fed over a longer period, resulting in greater loss of chlorophyll.

The chemical composition of plants is not only affected by abiotic factors (Germ *et al*., 2010) but may also be influenced considerably by biotic factors such as herbivory. The changes in pigment content due to feeding by *R. maidis* suggested a feeding induced response at different levels of infestation. Stress under aphid feeding led to lower chlorophyll and carotenoid content in sorghum plants. Huang *et al*. (2014) reported that the relative chlorophyll loss was related to the amount of feeding damage caused by insects and confirmed that this damage measured on individual host plants varied significantly depending on the insect density and stage of plant growth.

Photosynthetic pigment degradation is a complex phenomenon which often accompanies insect feeding damage to plants (Ni *et al*., 2002). According to Wilkaniec (1990), aphid feeding causes changes in the metabolism of host plants, which in turn disturbs photosynthesis, speeds tissue aging, and causes morphological deformations.

Table 4.8. Effect of different levels of aphid infestation on leaf pigments in sorghum

Attributes	Chl *a** (mg/g)	% reduction in Chl *a*	Chl *b** (mg/g)	% reduction in Chl *b*	Chl (*a*+*b*)* (mg/g)	% reduction in Chl (*a*+*b*)	Carotenoid* (mg/g)	% reduction in Carotenoid
No Infestation (Control)	25.9 ± 1.41^{a}	-	2.8 ± 0.23^{a}	-	28.7 ± 1.63^{a}	-	1.0 ± 0.04^{a}	-
Low Infestation	21.4 ± 1.94^{ab}	17.37	2.4 ± 0.34^{ab}	14.28	23.8 ± 2.25^{ab}	17.07	0.9 ± 0.05^{ab}	10
Medium Infestation	16.6 ± 1.19^{b}	35.90	1.5 ± 0.17^{b}	46.42	18.1 ± 1.30^{b}	36.93	0.7 ± 0.04^{b}	30
High Infestation	15.6 ± 2.74^{ab}	39.76	1.9 ± 0.41^{ab}	32.14	17.5 ± 3.09^{b}	39.02	0.6 ± 0.09^{b}	40
df	3, 25	-	3, 25	-	3, 25	-	3, 25	-
F value	5.64	-	3.15	-	5.38	-	6.25	-
P value	0.0043	-	0.0426	-	0.0054	-	0.0026	-

Means within a column followed by the same letter are not significantly different using Tukey's HSD test (α=0.05); df: degrees of freedom.

***(Mean±SEM)**

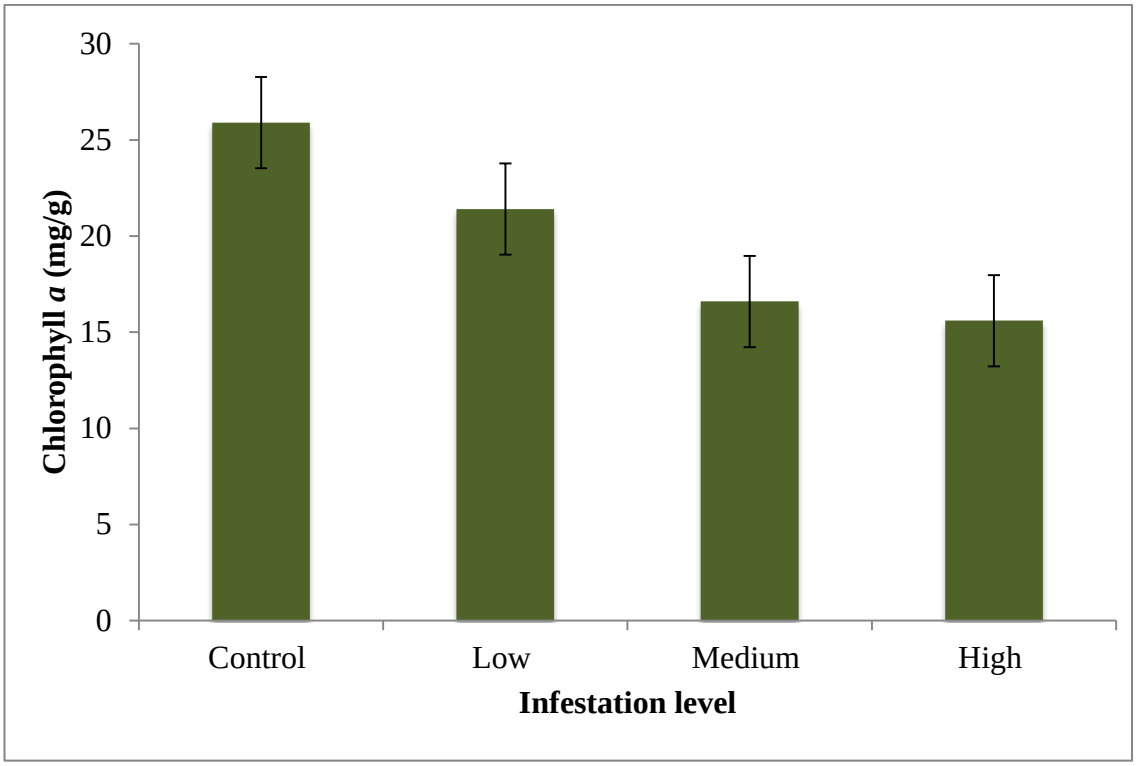


Fig. 9. Effect of different levels of aphid infestation on chlorophyll *a* concentration in sorghum

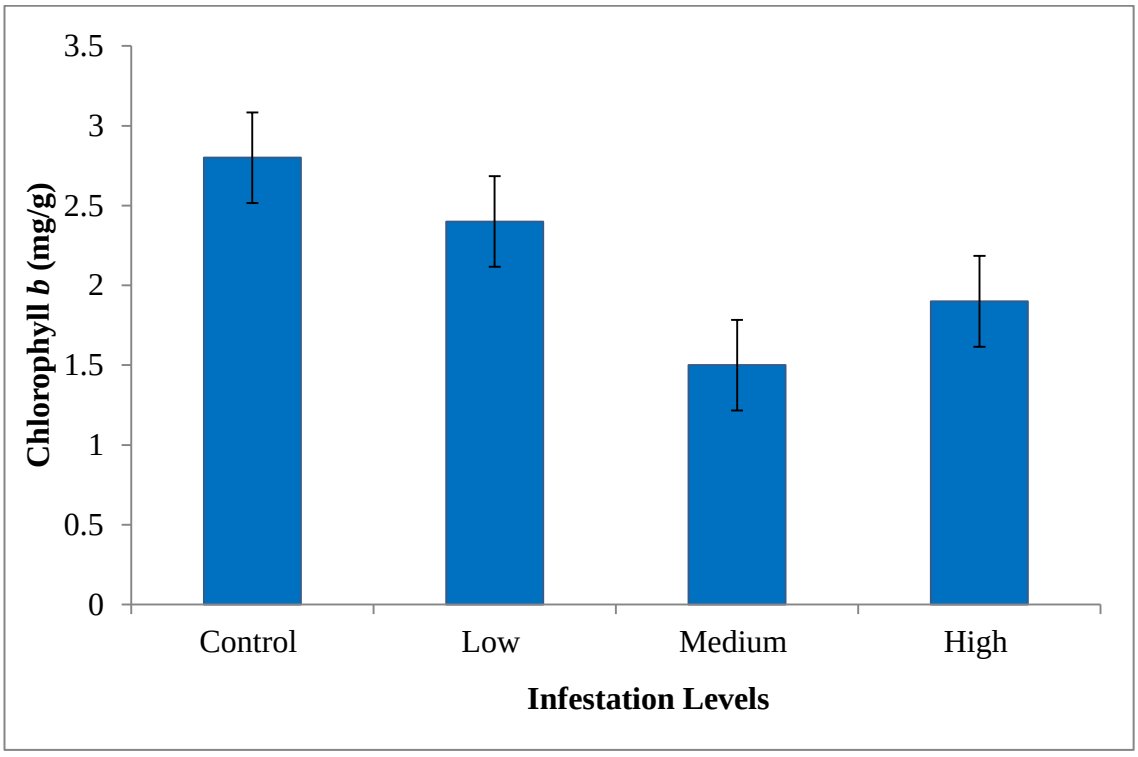


Fig. 10. Effect of different levels of aphid infestation on chlorophyll *b* concentration in sorghum

This is connected with the direct harm done by aphids inserting saliva into plant tissues and blocking stomata with the honeydew they produce.

The carotenoids serve as cellular membrane protectants and removal of carotenoid pigments may result in membrane degradation (Timko, 1998). Heng-Moss *et al.* (2003) stated that the reduction of chlorophyll *a* and *b* and carotenoid content after *D. noxia* feeding supported the suggestion by Fouche *et al.* (1984) that such herbivory negatively impacts the stacked region of thylakoid membranes. Carotenoids participate in light harvesting and protect the photosynthetic apparatus from photooxidative damage by quenching triplet state chlorophyll molecules and scavenging reactive oxygen species such as singlet oxygen (Biswal *et al*., 1994 and Malkin and Niyogi, 2000). Hence, the decreasing pattern of chl *a*, chl *b* and carotinoides was observed with infestation. The decrease in the photosynthetic pigments might be due to the inhibition of pigment biosynthesis which resulted from the alteration in mineral nutrition or lack of assimilates drain towards the insect or to the effect of reactive oxygen on these pigments (Stacey and Keen, 1996).

The phloem-feeding aphid continually controls and/or modifies the metabolic substances levels of the surrounding tissues. It was reported that strong and persistent flow of host assimilates created by the continual removal of metabolites and breakdown of insoluble reserves by insects (Kattab, 2005). John *et al.* (2007) explained that *A. glycines* removes phloem sap, which can result in a reduction of chlorophyll content. Hemmat Khattab (2007) showed the defence mechanism of cabbage plant against phloem sucking aphid. The levels of antioxidant compounds (like carotenoids) were changed in response to aphid feeding.

4.2.5.5 Flavonoid content

Aphid infestation also altered the flavonoid levels, which significantly increased under the stress of *R. maidis* infestation in tissues of sorghum leaves (Table 4.9 and Fig. 12). The plants with high infestation of aphids showed higher amount of flavonoid content *i.e.* 77.37±3.82 µg Rutin Equivalent (RE) compared to the control (38.24±3.32 µg RE). The aphid infested leaves showed significant ($F= 27.87$, $df = 8, 11$ and $P = 0.0001$) differences between the infested and un-infested leaves. The % increase in

flavonoid content over no- infestation was 24.21, 46.02 and 102.32 for various levels of infestation (low, medium and high infestation, respectively).

In our study, an increase of flavonoid content in tissues of the sorghum leaves *vis-a-vis* aphid density, under the stress of aphid feeding was clearly evident. Flavonoids are a large class of secondary metabolites encompassing more than 10,000 structures and are of great interest for their bioactivities, basically related to their antioxidant properties (Cao *et al.,* 2013). Several lines of evidence demonstrated that they have antioxidant functions in higher plants challenged with a range of environmental stresses (Winkel-Shirley, 2002 and Agati *et al.,* 2012). The stress reactions of the sorghum plants point to the negative effect of aphid infestation and to activation of protective mechanisms such as an increase of flavonoid content. Tevini *et al.* (1991) showed that accumulation of these pigments in rice lessened the damage to the photosynthetic activity of mesophyll chloroplasts. Flavonoids are generally involved in plant resistance to herbivores (Bennett and Wallsgrove, 1994 and Wu *et al.,* 2007). Leiss *et al.* (2009) showed that resistant hybrids contained higher amounts of the flavonoid kaempferol glucoside. Kaempferol glucosides also have a negative effect on aphids. Aphid-resistant cow pea lines contained significantly higher amounts of flavonoids, including kaempferol, than susceptible lines (Lattanzio *et al.,* 2000).

This study provided essential information on the effect of *R. maidis* feeding on chlorophyll, carotenoid and flavonoid contents in sorghum leaves. The extracted chlorophyll and carotenoid contents in medium and high infestation showed significant difference over no infestation while, in low infestation both the pigments were on par with no infestation. The flavonoid content increased as infestation level increased. Thus losses of chlorophyll and carotenoid concentrations and increased in flavonoid content in response to *R. maidis* infestation suggest a feeding-induced stress response in sorghum depending on the aphid density. The plant physiological parameter fluorescence and photosynthetic rate in low and medium infestation showed no significance difference over no infestation but, SPAD chlorophyll content in both infestation levels showed significance difference over no infestation with less amount of per cent reduction.

Table 4.9. Effect of different levels of aphid infestation on flavonoid content in sorghum

Attributes	Flavonoid content* (µg Rutin Equivalent)	% increase in flavonoid content
No Infestation (control)	38.24 ± 3.32^{a}	-
Low Infestation	47.95 ± 3.41^{ab}	24.21
Medium Infestation	55.84 ± 1.57^{b}	46.02
High Infestation	77.37 ± 3.82^{c}	102.32
df	8, 11	-
F value	27.87	-
P value	0.0001	-

Means within a column followed by the same letter are not significantly different using Tukey's HSD test (α=0.05); df: degrees of freedom.

***(Mean±SEM)**

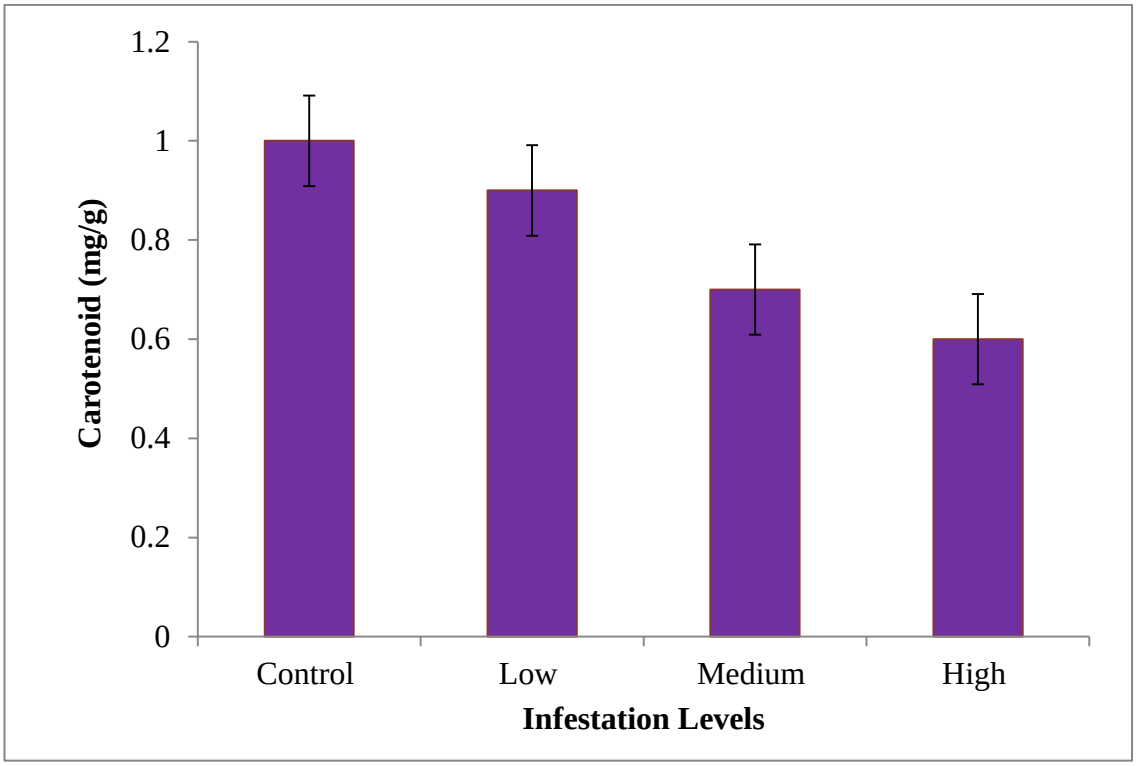


Fig. 11. Effect of different levels of aphid infestation on carotenoid concentration in sorghum

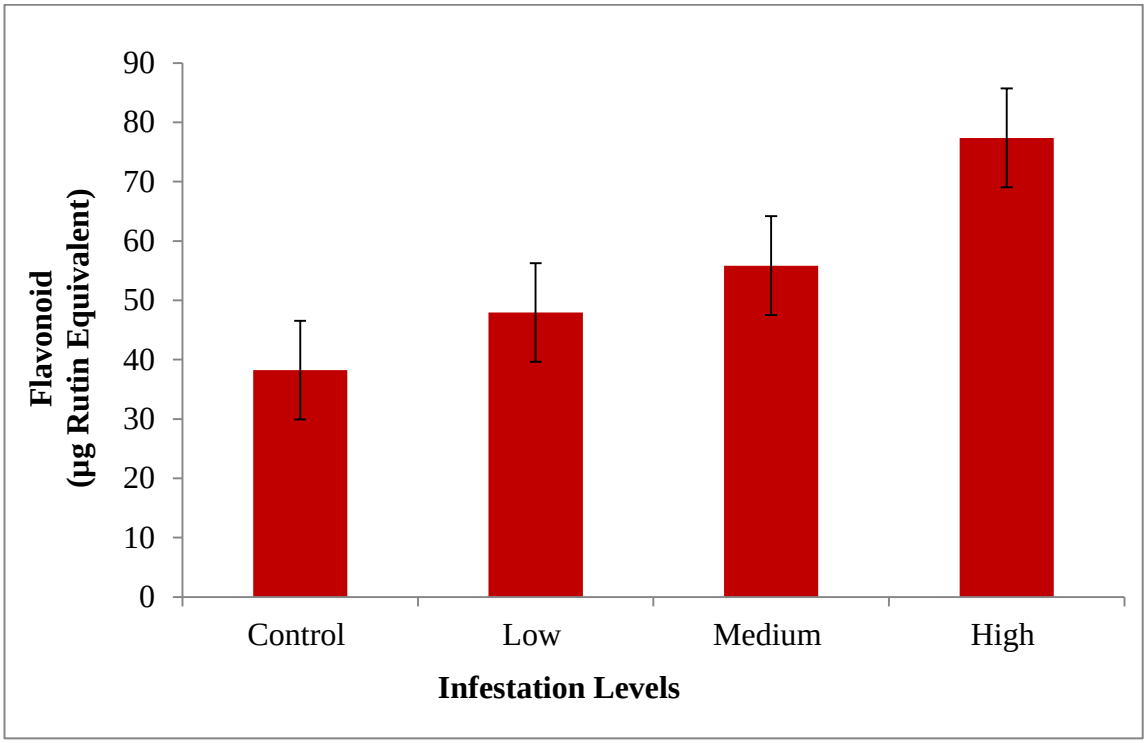


Fig. 12. Effect of different levels of aphid infestation on flavonoid concentration in sorghum

4.3 RELATIVE WATER CONTENT (RWC) IN SORGHUM DUE TO APHID FEEDING

Relative water content (RWC) decreased significantly in the affected plants compared to no infestation. The RWC in low and medium infestation (84.8±1.20% and 84.1±0.77%, respectively) showed lower RWC compared to high infestation (87.2±1.21%). However, the relative water content of low and medium infested plants showed significant difference with no infestation (89.3±1.20%) although the latter was on par with high infestation plants (Table 4.10).

Walter (1989) stated that leaves of barley plant infested with wheat aphid, *D. noxia* had lower relative water content than corresponding leaves from un-infested plants. This is similar to our findings where corn leaf aphid *R. maidis* infestation reduced RWC (from 89.3 % to 84.1%) in sorghum. Sanjay *et al.* (1984) showed that feeding injury caused by *E. dodecastigma* on bitter gourd leaves decreased the RWC varying from 96.5% to 78.0%.

In our study, in the aphid infested sorghum leaves, 2.35 to 5.82% reduction in RWC was noticed (Fig. 13). The significant reduction in RWC in cotton plants was 1.93 to 23.49% due to mealybug, *P. solenopsis* (Prabhakar *et al.,* 2013), 17% due to spider mite, *T. urticae* (Schmidt *et al.* 2009). Water-stress caused by greenbug infestation induces several metabolic changes that may be at nutritional-physiological level or behavioural responses, such as the length of time for feeding and preference for specific plants or location on plants (Zuniga *et al.,* 1989 and Holtzer *et al.,* 1988). Cornish and Zeevaart (1985) suggested that greenbug infestation could affect the regulation of water balance in leaves by affecting stomatal physiology. Cabrera *et al*. (1995) stated that barley seedlings infested with *S. graminum* have drought-stress symptoms, such as lower water potentials and lower relative water contents, even in the presence of ample root moisture.

Table 4.10. Effect of different levels of aphid infestation on relative water content (RWC) in sorghum

Treatment	Relative water content* (%)	% reduction in relative water content
No Infestation	89.3 ± 1.20^{a}	-
Low Infestation	84.8 ± 0.77^{b}	5.03
Medium Infestation	84.1 ± 0.86^{b}	5.82
High Infestation	87.2 ± 1.21^{ab}	2.35
df	3, 25	-
F value	5.64	-
P value	0.0043	-

Means within a column followed by the same letter are not significantly different using Tukey's HSD test (α=0.05); df: degrees of freedom.

***(Mean±SEM)**

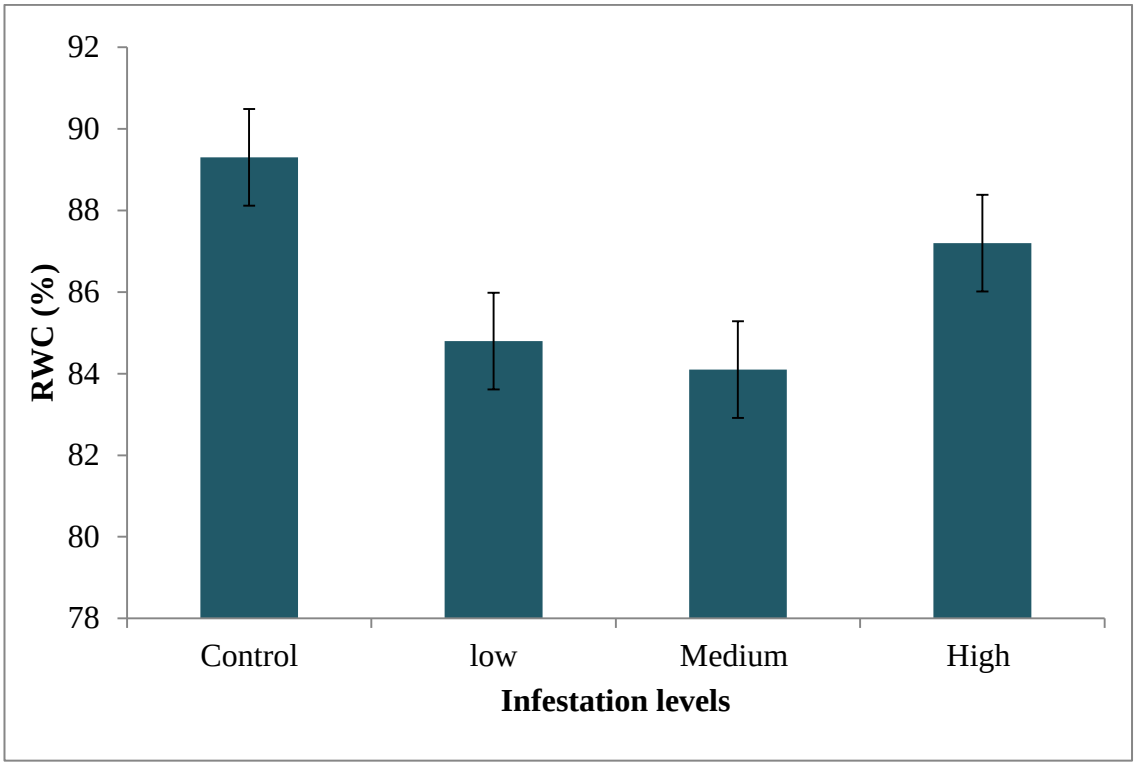


Fig. 13. Effect of different levels of aphid infestation on relative water content (RWC) in sorghum

In the present findings the per cent reduction in RWC was greater in low and medium infestation plants compared to high infestation. The probable reason could be that feeding by aphids in high infestation (300 aphids/plant) might have been effected due to overcrowding and competition as compared to low (100 aphids/plant) and medium infestation (200 aphids/plant). However, at high infestation there was a greater loss of leaf pigments, probably due to the compensation of pigments from the unaffected part of the leaf.

CHAPTER V

SUMMARY AND CONCLUSIONS

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Sorghum is attacked by many insect-pests which are the principal limiting factor for its productivity throughout the country. Leaf corn aphid, *Rhopalosiphum maidis* (Fitch) is one of the major insect pests which widely distributed and prevalent throughout India. To manage the aphids, adoption of ecologically safe strategy like Integrated Pest Management (IPM) is recommended. The development of effective and rational management of *R. maidis* relies on a thorough understanding of the biology of the pest and physiological losses caused by it. Keeping this in view, the present studies were undertaken on the biology of aphid, effect of insect density on plant physiological losses and relative water content (RWC) due to aphid feeding in sorghum.

The biology included studies on developmental period, longevity, fecundity, population growth statistics and morphometrics of corn leaf aphids reared on sorghum leaves in laboratory condition. The corn leaf aphid, *R. maidis* on sorghum had a life cycle of 7.79±0.12 days, adult longevity of 12.27±0.27 days and life span of 16.25±0.80 days. The fecundity and rate of reproduction were 35.97 progenies/female and 9.15 progenies/female/day, respectively. The net reproduction rate (R_0), mean generation time (T_c), intrinsic rate of natural increase (r_m), innate capacity of increase (r_c) and corrected mean generation time (T) were 45.05, 9.24, 0.44, 0.41 and 8.73 days, respectively.

The morphometrics data was measured by using stereo zoom microscope. The length and width of different stages of *R. maidis* were: 720.57± 9.95 µm and 303.62 ± 5.59 µm (1^{st} instar); 978.46 ± 13.51 µm and 412.83 ± 5.53 µm (2^{nd} instar); 1399.52 ± 24.56 µm and 581.74 ± 12.33 µm (3^{rd} instar); 1737.5 ± 20.28 µm and 733.98 ± 10.96 µm (4^{th} instar) and 1833.63 ± 15.89 µm in length and 783.26 ± 10.82 µm (adult), respectively.

Pot studies were undertaken to test the physiological losses caused by different levels of aphid density on sorghum plants. Sorghum plants raised in pots were infested with different density levels of aphids (low-100 aphids/plant; medium-200 aphids/plant and high infested-300 aphids/plant) at 25 days after sowing. The plant physiological

parameters *viz*., SPAD chlorophyll content, fluorescence and photosynthetic rate were recorded at 20 days after release by SPAD 502 meter (Soil Plant Analytical Development), Fluorometer and Infra-Red Gas Analyser, respectively.

The SPAD chlorophyll content under high, medium and low infestation (27.1±0.74, 33.4±0.39 and 35.1±0.44, respectively) showed significant difference over no infestation (38.6±0.60). *R. maidis* infestation on sorghum leaves showed 9.06-29.79% decline in chlorophyll content (SPAD units) over no infestation.

The lowest level of fluorescence (Fv/Fm) was noted in high infestation (0.69±0.01) and it showed significant difference over no infestation (0.74±0.01), while the medium (0.72±0.01) and low infestation (0.73±0.01) were on par with no infestation. The feeding of leaf corn aphids on sorghum leaves caused a decrease in the Fv/Fm level to a minimum value of 1.35% (low infestation) to a maximum of 6.75% (high infestation) compared to the control.

The photosynthetic rate (µmol CO_2 m^{-2} sec^{-1}) in sorghum plants with high infestation was 13.2±1.23 which was significantly different from no infestation (19.5±1.02). However, there was no much variation in photosynthetic rates of low (18.3±0.6) and medium infested plants (17.6 ±0.65).

A negative correlation between aphid density and physiological parameters indicated that an increase in the incidence of *R. maidis* (density) decreased the chlorophyll content, fluorescence and photosynthetic rate. A significant reduction in all the three physiological parameters were recorded at high density level of aphids on sorghum.

After taking reading of physiological parameters, the loss in leaf pigments *viz.,* chl *a*, chl *b*, chlorophyll (*a*+*b*), carotene and flavonoids were quantified by extraction method. It was found that low infestation of *R. maidis* on sorghum leaves caused over 17.37% and 39.76% (high infestation) decrease in chl *a* compared to no infestation. The medium infestation (16.6±1.19 mg/g) showed highly significant difference over no infestation (25.9±1.41 mg/g).

The lowest content of the chl *b* (1.5±0.17 mg/g) was found in leaves infested with 200 aphids/plant (medium infestation) and it showed significant difference (2.8±0.23 mg/g) over no infestation but was on par with low (2.4±0.34 mg/g) and high (1.9±0.41 mg/g) infestation.

The chl (*a*+*b*) content in medium (18.1±1.30 mg/g) and high infestation (17.5±3.09 mg/g) showed significant difference over no infestation (28.7±1.63 mg/g). However, the chl (*a*+*b*) in low infestation (23.8±2.25 mg/g) was on par with no infestation.

The lowest level of the carotenoid content was noted in high infestation *i.e.*, 0.6±0.09 mg/g of the leaf. The aphid infested leaves showed significant differences in carotenoid content between the infested (medium and high) and un-infested leaves.

The plants with high infestation of aphids showed higher amount of flavonoid content *i.e.*, 77.37±3.82 µg Rutin Equivalent (RE) compared to the control (38.24±3.32 µg RE). The aphid infested leaves showed significant differences between the infested and un-infested leaves. The % increase in flavonoid content over no infestation was 24.21, 46.02 and 102.32 for various levels of infestation (low, medium and high infestation, respectively).

For estimation of relative water content (RWC) the fresh weight (FW), turgid weight (TW) and dry weight (DW) were recorded. Relative water content (RWC) decreased significantly in the affected plants compared to no infestation. The RWC in low and medium infestation (84.8±1.20% and 84.1±0.77%, respectively) showed lower RWC compared to high infestation (87.2±1.21%). However, the relative water content of low and medium infested plants showed significant difference with no infestation (89.3±1.20%) although the latter was on par with high infestation plants.

Hence, it can be concluded that:-

- At 27^{o}C, the developmental period of *R. maidis* was 7.79±0.12 days, while the total life span was 16.25±0.80 days.
- Low infestation (100 aphids/plant) affected chlorophyll content, medium infestation (200 aphids/plant) affected chlorophyll and photosynthetic rate while, high infestation (300 aphids/plant) affected all the three parameters significantly.
- The extracted chlorophyll and carotenoid concentrations decreased significantly at medium and high infestation.
- Flavonoid content increased as infestation level increased.
- The per cent reduction in RWC was greater in low and medium aphid infestation.

The present study focuses on need to understand the relationship between insect densities and there effect on various physiological parameters before formulating sound strategies for pest management.

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