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Host plant resistance in maize hybrids to fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) (J.E. Smith)

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Abstract
The antibiosis and antixenosis in different ruling maize hybrids *viz*., Pioneer 3401, NMH 3095, C.P.818 and the TNAU maize hybrids CO 6, COH(M)8 to fall armyworm (*Spodoptera frugiperda)* were assayed by evaluating insect growth parameters like developmental period and weight of different stages, nutritional parameters like ECI, ECD, AD and trichome density. Developmental period for larva, pre pupa and pupa was shortest on the hybrid P3401 (13.86 ± 0.26, 1.24 ± 0.01 and 6.09 ± 0.19 days) and longest on CO 6 (17.5 ± 0.1, 1.79 ± 0.02 and 8.27 ± 0.03 days). The weight of the 3rd, 4th, 5th and 6th instar larva, pre pupa and pupa was maximum on P3401 (72.22 ± 1.08, 142.13 ± 3.38, 281.26 ± 5.86, 354.68 ± 4.45, 323.2 ± 6.37 and 286.3 ± 5.25 mg) and minimum on CO 6 (57.28 ± 1.13, 100.27 ± 1.38, 259.33 ± 6.25, 310.22 ± 4.75, 274.9 ± 5.79 and 232.6 ± 4.74 mg). The nutritional indices namely ECI, ECD and AD were maximum on P3401 (53.28 ± 1.23, 96.11 ± 1.63 and 55.43 ± 1.62) and minimum on CO 6 (49.03 ± 1.00, 92.24 ± 2.76 and 53.16 ± 0.97). The soluble protein and anthocyanin in leaves were maximum in COH(M) 8 (68 mg/g and 0.604 mg/100g) and minimum in P3401 (39 mg/g and 0.416 mg/100g), total phenol and chlorophyll were maximum in CO 6 (13.475 mg/g and 1.316 mg/g) and minimum in NMH 3095 (5.415 mg/g and 0.675 mg/g).

Keywords: Host plant resistance, fall armyworm, antibiosis, antixenosis, growth parameters

Introduction
Maize (*Zea mays* Linn.) is one of the most versatile emerging crop having wider adaptability and grown in diverse conditions and seasons. In India it is cultivated in 90.27 lakh ha with the productivity of 3070 kg/ha (INDIASTAT, 2019). The versatile use and low per unit cost of production make the maize as unique crop (Tariq, 2010) [23]. Though around 250 insect species are associated with maize in field and storage conditions (Mathur, 1992) [12], the cultivation is threaded by the invasive fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in recent days. In India it was first reported from Karnataka during May, 2018 (Sharanabasappa *et al.*, 2018) [2]. It is polyphagous and has been reported on 353 different plant species and has preference towards the grass family (Montezamo *et al.*, 2016) [14]. It causes huge economic losses in variety of crops such as maize, soybean, cotton, beans, sunflower, groundnut, tobacco, chillies, pulses *etc*., (Goergen *et al*., 2016) [6]. In the absence of management practices it can cause up to 20.6 million tonnes of maize yield loss per year (Day *et al*., 2017) [4]. As *S. frugiperda* is an introduced pest to India, the farmers solely depend on the chemical insecticides which are unsustainable and untenable in long run owing to their negative impact on the environment and possible resistant development. So there is an urgent need to develop safe and sustainable management strategies to manage this pest. Host plant resistance is one of the best and sustainable component in the IPM strategy. Among the host plant resistance mechanism, antibiosis plays a significant role against the gregarious pest like *Spodoptera frugiperda* (Kennedy *et al*., 1987) [8]. Nutritional and secondary metabolite variations among the maize hybrids influence the feeding preference of fall armyworm. Hence this study will help to formulate suitable and cost effective integrated pest management (IPM) packages for *S. frugiperda* on maize.

Materials and Methods
Collection and rearing of *S. frugiperda*
The life stages of fall armyworm were collected from the infested maize field of Cotton

Research Station (CRS), Veppanthattai, Tamil Nadu. The larval population collected from the field was reared at Entomology laboratory, Anbil Dharmalingam Agricultural College and Research Institute, Trichy at room temperature. The neonate larvae were reared on tender maize leaves, in an individual plastic container of 5 cm diameter. The adults were fed with honey solution (5 ml honey, 500 ml water, 3 vitamin E tablet, 30 g sucrose, 0.5 g methyl paraben, 0.5 g ascorbic acid) and mass reared in oviposition cage (30 × 30 cm).

Life cycle study

Five ruling maize hybrids (Pioneer 3401, NMH 3095, C.P.818 and the TNAU maize hybrids CO 6, COH(M)8) were grown in plastic pots under greenhouse condition. First instar larvae were released on ten days old maize plant had 6 to 7 expanded leaves. Larvae were released near the whorl region and allowed until pupation. Once in a week or whenever necessary maize plants were changed based on damage and the larva from damaged plant was collected and released on the replaced plants. Experiment was conducted by using randomized block design with five treatments and three replications with ten plants per replication. For avoiding the larval movement from one plant to another plant, each plant was covered with mylar sheet. Larvae were observed once in 24 hours to record the larval duration and larval weight.

After attained the fourth instar stage, larvae collected from the plant and reared on separate plastic cups and fed with maize hybrid leaves to record the food consumption rate as later larval instars were consumed more food. To study the growth indices, sixth instar larvae were fed with measured amount (0.5 g) of maize leaves for two days. The larval weight before and after feeding, weight of faeces and weight of left over leaves were taken at 12 hours interval to determine the Efficiency of conversion of ingested food (ECI), Efficiency of digested food (ECD) and Approximate digestibility (AD) (Schroeder, 1971) [20]. Nutritional indices of *S. frugiperda* were recorded by using the equation I = P +M +E given by Waldbauer (1968) [24].

ECI = 100P/I

P = Growth rate, GR = Wf - Wi/WeT

Where,

We = Mean of 12 hours interval weights gained by larvae

Wi = Weight of larvae before feeding

Wf = Weight of larvae after feeding

F = Weight of faecal matter

T = Duration of instar (days)

AD = 100(I-E)/I

ECD = 100P/I-E

I = Consumption index, CI = C1- C2/WeT

E = Faecal matter

Where,

C1 = Weight of leaves given

C2 = Weight of uneaten leaves

Biophysical resistance

The trichome density on the above five maize hybrids was counted. One square centimeter grid was cut in a card board. The grid was kept on the upper side of maize leaves. Number of trichomes present in one square cm was counted under Phase contrast microscope and expressed as number of trichomes per cm^2.

Biochemical analysis

Total soluble protein and secondary metabolites were analyzed in the leaves of above maize hybrids. Total soluble protein at 660 nm (Lowry *et al*., 1951), Total phenolics at 660 nm (Malik and Singh, 1980), total chlorophyll at 652 nm, anthocyanin content at 525 nm (Swan and Hillis, 1959) were estimated through spectrophotometer reading.

$$\text{Total Soluble Protein} = \frac{x}{1} \times \frac{25}{250} \times 100 \text{ mg/g of leaf sample}$$

$$\text{Total phenol} = \frac{X \times 25 \times 1000}{1 \times 500} \text{ mg/g of leaf sample}$$

$$\text{Total Chlorophyll} = \text{Absorbance}_{652} \times \frac{V}{1000 \times W} \text{ (per g tissue)}$$

$$\text{Concentration of Anthocyanin} = \frac{\text{Absorbance} \times \text{Volume of extraction solution} \times 100}{\text{Weight of sample} \times 98.2} \text{ (mg/100 g of leaf)}$$

Results and Discussion

Biology of *S. frugiperda*

Biology of fall armyworm was studied on five different maize hybrids. Total larval period was shorter on the hybrid P3401 (13.86 ± 0.26 days) followed by the hybrid NMH 3095 (14.37± 0.30 days) and longer on the hybrid CO 6 (17.5 ± 0.1 days). The developmental period of first, second, third, fourth, fifth and sixth instar larvae was 2.51 ± 0.05, 2.13 ± 0.03, 1.92 ± 0.05, 1.96 ± 0.04, 2.16 ± 0.05 and 3.18 ± 0.01 days respectively on the hybrid P3401. Correspondingly it was 3.02 ± 0.07, 2.37 ± 0.03, 2.45 ± 0.06, 2.68 ± 0.08, 3.06 ± 0.09 and 3.92 ± 0.06 days on the hybrid CO 6 (Table 1). This indicated the variation in the developmental period of the fall armyworm larvae when fed with the different maize hybrids. Liu *et al*. (2004) [15] proved that, the host plant species have vital function on developmental period of insects. Variations in larval period of beet armyworm *Spodoptera exigua* on different soybean varieties were demonstrated by Bernays and Chapman, (1994) [1]. In our study, life cycle of FAW varied from 27.69 days (P3401) to 34.56 days (CO 6) which was similar to the findings of Mardani *et al.* (2012) [11] on life history studies of *S. exigua* on corn hybrids. De La Rosa *et al*. (2015) [5] reported that life cycle of *S. frugiperda* larvae was longer on Tuxpeno variety than Pioneer 4063W maize variety. Duration of pre pupa (1.24 ± 0.01), pupa (6.09 ± 0.19 days) were shorter on P3401 and longer on CO 6 (1.79 ± 0.02) and (7.1 ± 0.61 days). In contrast, the adult longevity was longest when fall armyworm fed with P3401 (9.1 ± 0.27) and shortest when fed with CO 6 (7.1 ± 0.61 days) (Fig 1).

Table 1: Development of *S. frugiperda* on different maize hybrids

Maize hybrids	Larval duration (days)						Total
	1St instar	2nd instar	3rd instar	4th instar	5th instar	6th instar	
P3401	2.51±0.05 (1.58)e	2.13±0.03 (1.45)d	1.92± 0.05 (1.38)d	1.96± 0.04 (1.4)e	2.16± 0.05 (1.46)d	3.18± 0.01 (1.78)e	13.86± 0.26 (3.72)e
NMH3095	2.62±0.07 (1.61)d	2.22± 0.05 (1.48)c	2.00± 0.02 (1.41)c	2.02± 0.06 (1.42)d	2.20± 0.03 (1.48)d	3.31± 0.04 (1.81)d	14.37± 0.30 (3.79)d
CO6	3.02± 0.07 (1.73)a	2.37± 0.03 (1.53)a	2.45± 0.06 (1.56)a	2.68± 0.08 (1.63)a	3.06± 0.09 (1.74)a	3.92± 0.06 (1.97)a	17.5 ± 0.1 (4.18)a
COH(M)8	2.79± 0.07 (1.67)b	2.34± 0.01 (1.52)ab	2.39± 0.01 (1.54)a	2.46± 0.02 (1.56)b	2.96± 0.09 (1.72)b	3.64± 0.07 (1.90)b	16.58± 0.22 (4.07)b
C.P.818	2.70±0.01 (1.64)c	2.30± 0.05 (1.51)b	2.22± 0.07 (1.48)b	2.28± 0.06 (1.50)c	2.48± 0.04 (1.57)c	3.43± 0.12 (1.85)c	15.41± 0.09 (3.92)c
SEd	0.01**	0.0075**	0.0095**	0.0065**	0.01**	0.01**	0.028**
CD	0.021	0.016	0.020	0.013	0.021	0.024	0.061
CV%	0.80	0.64	0.82	0.55	0.83	0.80	1.02

Note: Each value is a mean of four replication with standard deviation (Mean ± SD)
Figures within parentheses are square root transformed values

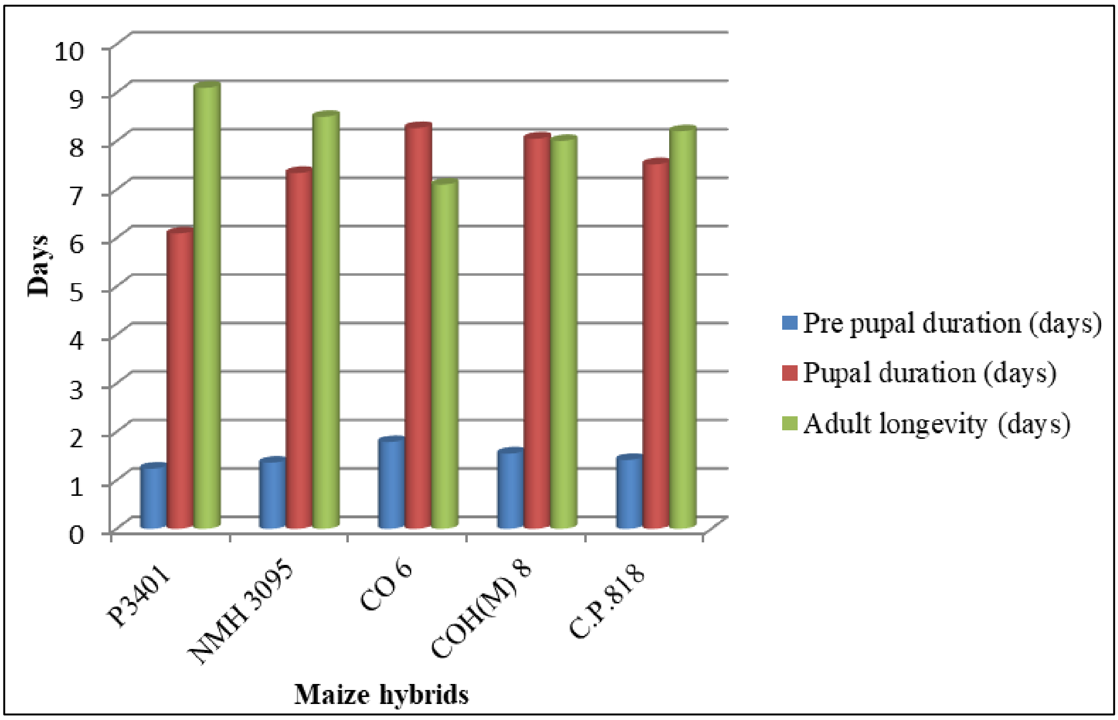

Fig 1: Pupal period and adult longevity of *S. frugiperda* feeding on different maize hybrid

Weight of developmental stages of *S. frugiperda*

There was a significant difference among the weight of the different instars fed with different maize hybrids. Maximum weight of third instar 72.22 ± 1.08 mg, fourth instar 142.13 ± 3.38 mg, fifth instar 281.26 ± 5.86 mg and sixth instar 354.68 ± 4.45 mg was observed on the hybrid P3401 and the weight was minimum on the hybrid CO 6 (57.28 ± 1.13 mg) for third instar, 100.27 ± 1.38 mg for fourth instar, 259.33 ± 6.25 mg for fifth instar and 310.22 ± 4.75 mg for sixth instar. The pre pupal weight was also maximum on P3401 (323.2 ± 6.37 mg) and minimum on CO 6 (274.9 ± 5.79 mg). Similarly, P3401 recorded the maximum pupal weight (286.3 ± 5.25 mg) and CO 6 recorded the minimum pupal weight (232.6 ± 4.74 mg) (Table 2). This indicated that, CO 6 showed some resistance to fall armyworm. Leuck and Perkins (1972) [9] used the pupal weight of the lepidopteran insects as a fitness indicator. The quality of food can have an effect on the growth of insects (Slansky and Scriber, 1981) [22].

Table 2: Weight of developmental stage of fall armyworm feeding on different maize hybrids

Maize hybrids	Larval weight (mg)				Pre pupal weight (mg)	Pupal weight (mg)
	3rd instar	4th instar	5th instar	6th instar		
P3401	72.22±1.08 (8.49)a	142.13±3.38 (11.91)a	281.26±5.86 (16.77)a	354.68±4.45 (18.83)a	323.2±6.37 (17.97)a	286.3±5.25 (16.92)a
NMH3095	69.06±0.75 (8.31)b	132.02±1.76 (11.48)b	269.81±5.48 (16.42)b	334.33±5.45 (18.28)b	318.2±6.92 (17.83)a	273.4±1.11 (16.53)b
CO6	57.28±1.13 (7.56)e	100.27±1.38 (10.01)e	259.33±6.25 (16.10)c	310.22±4.75 (17.61)d	274.9±5.79 (16.58)b	232.6±4.74 (15.25)d
COH(M)8	59.93±0.65 (7.74)d	110.43±3.34 (10.50)d	261.12±6.09 (16.15)c	322.61±2.21 (17.96)c	282.6±4.42 (16.81)b	241.7±2.63 (15.54)c

C.P.818	66.48±1.90 (8.15)[c]	122.56±2.52 (11.07)[c]	264.90±5.41 (16.27)[c]	330.42±4.42 (18.17)[bc]	314.4±6.41 (17.73)[a]	268.2±5.29 (16.37)[b]
SEd	0.039**	0.080**	0.120**	0.145**	0.129**	0.114**
CD	0.084	0.172	0.255	0.309	0.276	0.244
CV%	0.70	1.04	1.04	1.13	1.06	1.01

Note: Each value is a mean of four replication with standard deviation (Mean ± SD)
Figures within parentheses are square root transformed values

Growth indices for *S. frugiperda*

Efficiency of conversion of ingested food (ECI) was maximum in P3401 (53.28 ± 1.23) and minimum in CO 6 (49.03 ± 1.00). Efficiency of digested food (ECD) was maximum in P3401 (96.11 ± 1.63) which was on par with NMH 3095 (95.43 ± 1.10) and C.P.818 (94.32± 2.11) and it was minimum in CO 6 (92.24 ± 2.76). Approximate digestibility (AD) was maximum in P3401 (55.43 ± 1.62) which was on par with COH(M) 8 (54.14 ± 1.10) and C.P.818 (54.24 ± 0.03) and minimum in CO 6 (53.16 ± 0.97) and NMH 3095 (53.62 ± 1.20) (Table 3). Lowest efficiency of conversion of food into body mass resulted in reduced growth (Slansky and Scriber, 1985) [22]. In our study also lowest ECI and AD observed on CO6 (49.03 ± 1.00) and (53.16 ± 0.97%) and developmental period was longer. The lowest rate of ingestion and conversion of food indicates, larvae spent longer time to attain growth. The calculation of food consumption may be used for economic threshold data development in pest management studies.

Table 3: Growth indices for *S. frugiperda* feeding on maize hybrids

Hybrids	ECI	ECD	AD
P3401	53.28 ± 1.23a	96.11 ± 1.63a	55.43 ± 1.62a
NMH3095	51.18 ± 0.62b	95.43±1.10ab	53.62 ± 1.20b
C06	49.03 ± 1.00c	92.24 ± 2.76c	53.16 ± 0.97b
C0H(M)8	50.83 ± 1.03b	93.88±1.72bc	54.14±1.10ab
C.P.818	51.16 ± 1.13b	94.32± 2.11abc	54.24 ± 0.03ab
SEd	0.307**	1.344*	0.401*
CD	0.654	2.864	0.854
CV%	0.95	2.47	1.19

Biophysical and Biochemical content of maize hybrids

Biophysical characters and biochemical content of the host plants play a vital role in exerting the host plant resistance on insect pests. Trichome density was maximum in NMH 3095 ($26/cm^2$) followed by CO 6 ($24/cm^2$) (Table 4) among the different maize hybrids. In plants trichomes evolved a defensive function and preventing the attack of pests and gives fitness advantage. We studied the trichome density of maize hybrids and its influence on feeding, consumption and digestibility by *S. frugiperda*. These physical defenses had prolonged the life cycle and development of fall armyworm. Density of trichomes plays a crucial role in plant resistance and had an influence against the chewing damage by *S. frugiperda* (Gustavo Moya-Raygoza *et al.*, 2016) [16]. Trichomes interfere with the insect digestion (Wellso, 1973) [25].

Soluble protein content was maximum in hybrids COH(M)8 (68 mg/g) and CO 6 (61 mg/g) and minimum in P3401 (39 mg/g). Greater soluble protein in the plant system results in excessive RuBisCO (Ribulose-1,5-bisphosphate Carboxylase) activity, which in long run induce more carbon fixation leads to high photosynthetic efficiency. The maximum amount of non-defensive soluble protein and minimum induction of defensive proteins in the susceptible plants leads to faster growth of larvae (Mattson, 1980) [13]. In susceptible corn tissues the amino acids, asparagines and proline was higher. Protein content in plants did not have much effect on larval growth (Hedin *et al*., 1989) [7].

Highest phenolic content was observed on CO 6 (13.475 mg/g) which showed more resistance than other hybrids and lower in NMH 3095 (5.415 mg/g) which is comparatively much susceptible. Phenolics present in plant parts offered chemical defense against insect herbivores and had direct or indirect effect on growth of herbivores (Pechan *et al.*, 2000) [17]. High level of polyphenols may reduce the feeding and growth of larvae (Davis *et al.*, 1995) [3]. Response of maize to mediterranean corn borer attack was mediated by jasmonic acid (Rogelio Santiago *et al.*, 2017) [18]. Some phenolic compounds are responsible for Systemic Acquired Resistance. Presence of chloronergic acid (phenolic acid) in maize act as an antifeedant against European corn borer (Mao *et al*., 2007) [10].

Total chlorophyll content was higher in CO 6 (1.316 mg/g of leaves) followed by COH(M)8 (1.252 mg/g of leaves) and lower in NMH 3095 (0.675 mg/g of leaves). Initially chlorophyll was used by plants for photosynthesis which in turn resist the leaf consuming pests. Chlorophyllide produced by insect feeding binds to insect gut in greater than chlorophyll. Plants converting chlorophyll into chlorophyllide and guard themselves from herbivore damage. When those leaves had been given to larvae of *Spodoptera litura*, the growth became suppressed and mortality rate was increased after ingestion (Ryouichi ranaka, 2015).

High level of anthocyanin content recorded from COH(M)8 (0.604 mg/100g of leaf) followed by CO 6 (0.592 mg/100g) and lower in P3401 (0.416 mg/100g). Anthocyanins help plants in their defense against herbivores. These have antiviral, antibacterial and fungicidal activities. Flavonoid includes anthocyanin and tannins act as a feeding deterrent against *S. frugiperda* (Singh *et al*., 2020) [21]. Larvae analyses the chemical and physical tendencies of plant during feeding and decide to select or reject the food (Schoonhovan *et al*., 2007) [19].

Table 4: Biophysical and Biochemical content of different maize hybrids

Maize hybrids	Trichomes/ cm^2	Soluble protein (mg/g)	Total phenolics (mg/g)	Chlorophyll (mg/g)	Anthocyanin (mg/100g)
P3401	19	39	6.365	0.707	0.416
NMH3095	26	48	5.415	0.675	0.462
C0 6	24	61	13.475	1.316	0.592
COH(M)8	21	68	6.365	1.252	0.604
C.P.818	12	53	6.365	0.936	0.497

Conclusion

In this current study, *S. frugiperda* cultures were raised in various maize hybrids leaves for studying its lifecycle. We identified the physiological differences and preference of fall armyworm fed with the hybrids of same host. Host plant resistance is a crucial tool for pest control strategies. The amount of food consumed by an insect can have an effect on its survival, growth and reproductive capacity. We suggest that, shorter developmental time, heaviest larval and pupal weight observed on Pioneer 3401 compared with other maize hybrids selected for experimental studies. The longer developmental time and lightest larval and pupal weight observed on CO 6, the decreased suitability of this hybrid may be because of the presence of few phytochemicals performing as antibiotic agents or absence of some primary nutrients critical for development of *S. frugiperda*. We did not locate complete resistance to larval feeding in the maize hybrids.

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