Effect of Insecticides on Feeding Behavior, Food Consumption and Survival of Spodoptera frugiperda (Lepidoptera: Noctuidae)

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Abstract The fall armyworm, Spodoptera frugiperda Smith (Lepidoptera: Noctuidae), is an invasive insect pest attacking maize in Pakistan. In the present study, we observed the effect of insecticides on feeding behavior, food consumption and survival of S. frugiperda. Chlorantraniliprole (125 ml/ha), Spinetoram (200 ml/ha), Emmamectin benzoate (500 ml/ha), Indoxacarb (440 ml/ha), Lambda-cyhalothrin (620 ml/ha), Flubendiamide (60 ml/ha) and Novaluron (750 ml/ha) concentrations were used to observe super-lethal, lethal, median-lethal and sub-lethal effects. Results revealed that Chlorantraniliprole showed the highest mortality (94%) followed by emamectin benzoate and flubendiamide (79%), spinetoram (77%), lambda-cyhalothrin (71%), indoxacarb (68%) and novaluron (23%). It was observed that an increase in concentrations was directly proportional to mortality, but a decrease in concentrations led to a decrease in mortality. No feeding rate or food consumed by larvae due to the highest toxicity of chlorantraniliprole but in the case of novaluron maximum food consumed by larvae due to less toxicity level. The result indicates that insecticide formulations had a significant effect on 2^{nd} instar larval mortality, food consumption, weight gain, development time, the weight of ingested food (WIF), relative consumption Rate (RCR), final weight gain (FWG), relative growth rate (RGR), the weight of feces, approximate digestibility (AD), ingested food to body substance (ECI), the efficiency of conversion of digested food into growth (ECD) and assimilation rate (AR). Maximum larvae survival and food consumption and weight were observed in control. In the case of super-lethal and lethal doses of all treatments, S. frugiperda larvae showed maximum mortality and less food consumption as compared to median-lethal and sub-lethal treatments.

Keywords Fall armyworm, Mortality, Insecticides, Feeding, Survival.

I. INTRODUCTION

aize is the third most important cereal grain after wheat and rice globally, which is also called the "Queen of Cereals" because of its highest genetic yield potential [1]. Maize is being grown on an area of 1.016 million hectares with an annual production of 3.037 million tons and an average grain yield of 2,864 kg/ha [2]. The contribution of maize to the total value added in agriculture remained at 2.1% and to the GDP at 0.4% from 2013 to 2014. It was planted at an area of 1.12 million hectares (65% irrigated, and 35% rainfed) resulting in a production of 4.53 million tons. Maize production worldwide, Pakistan comes at 17th place in the ranking [3]. Maize is traditionally a summer crop grown mainly in the two provinces, Khyber Pakhtunkhwa and Punjab. The introduction of hybrid maize, particularly planted in the spring season in Punjab, with yields averaging 8-9 t ha⁻¹ has revolutionized maize production [4]. In Pakistan, per hectare yield of maize has not increased despite the introduction of high-yielding varieties, the major obstacle in achieving this goal is the attack/infestation by insect pests. The notable insect pests are maize and sorghum stem borer (*Chilo partellus*) and shoot fly (*Atherigona soccata*) the infestation of which ultimately results in total failure of autumn and spring crops, respectively [5]. Numerous insect pests cause damage to maize crop but the fall armyworm (FAW), Spodoptera frugiperda Smith (Lepidoptera: Noctuidae) is a major pest of the maize field. This pest is polyphagous and found in several countries such as Brazil, Argentina, and the USA [6], causing economic losses in a variety of crops such as maize Zea mays, soybean Glycine max, cotton Gossypium hirsutum and beans *Phaseolus vulgaris* [7].

The fall armyworm, *S. frugiperda* has been classified as a sporadic pest due to its migratory behavior. This species does not enter diapause, so it migrates from warmer climates such as southern Florida, the Caribbean Islands, southern Texas, Mexico, and coastal areas of southern Georgia, Alabama, Mississippi, and Louisiana, northward across the United States annually [8]–[12]. The pest is native to tropical and subtropical regions of the Americas and

is the key insect pest of maize in tropical [13]. Recently, *S. frugiperda* has reached the African continent, with its first reported appearance in Nigeria in 2016 and it has spread to more than 28 countries in Southern and Eastern Africa [14]. Most recently, it has been reported from many maize-producing areas of India, Yemen, Thailand, Sri Lanka, Bangladesh, Myanmar and China. Since its introduction in Africa, it had caused severe economic losses amounting to millions of dollars [15]. Being distributed to 40 sub-Saharan African countries, the pest has already migrated to India where the first incidence was seen in July 2018 [16].

Fall armyworm, *S. frugiperda* is a globally declared polyphagous pest its hosts exceed 80 plant species including maize, sorghum, cotton, rice, millet, peanut, alfalfa, and other cultivated and wild plant species [17] and in particular, is threatening maize production systems worldwide. This pest can cause a 70% yield reduction when maize plants are attacked during the early stages [18]. It has become an invasive, and quarantine pest which has been affecting more than 200 million people whose staple crop is maize. In the absence of proper control measures, *S. frugiperda* has the potential to cause 8.3 to 20.6 million tonnes of maize yield losses per annum in Africa. The value of crop losses is estimated between US\$ 2.5 and 6.2 billion [19]. Larvae of *S. frugiperda* can cause high damage to the crop which includes loss of photosynthetic area, direct damage to grain, lodging and structural damage in the whorl in the maize plant [20]. However, the sector's contribution to food security and poverty reduction is limited by many, often interacting, biotic and abiotic factors. For example, the recent invasion of fall armyworm has become a major threat to food security in the region [19]. The fall armyworm is an invasive and damaging pest native to tropical and subtropical America, but it is spreading across Africa.

The fall armyworm *S. frugiperda* is considered a major pest of tropical-subtropical origin in the Western Hemisphere ranging from Argentina to southern Canada [21]. Because of its wide host range, *S. frugiperda* is one of the most harmful pests threatening annual crops in tropical regions [22]. Control of this pest is challenging because possible host plants have different phenologies and are grown during different seasons of the year though in proximity to each other, which can facilitate movement of the pest between crops. This availability of different hosts might even result in the selection of insect populations with new food preferences due to different exposure of these insects to a variety of crops [23]. Although primary damage to the foliage is done by the younger larvae and major reason for the reduction in yield and quality of the maize grains is due to the feeding of the cob and kernels by the larger larvae that are present in the whorls of older plants. Reports indicated that the estimated national mean loss of maize in Ghana was 45% (22-67%), and in Zambia 40% and it is estimated that without control measures. Fall armyworm is expected to reduce maize yield by 8.3 to 20.6 million tons per year of the total expected production of 39 million tons per year [19]. Leaf, silk and tassel damage levels range between 25 and 50% and grain yield decreases by 58% [20].

It has been reported to infest >80 plant species, including important crops such as cotton, soybean, and corn [24]. In Brazil, the fall armyworm is considered the most destructive and economically important pest in corn [25]. In the United States, it has been described as an important yield-limiting pest in southern cornfields [26]. This species does not diapause over winter, being vulnerable to low winter temperatures, only surviving year-round in the subtropical climates in the southern regions of Florida and Texas [27]. Therefore, *S. frugiperda* populations migrate and reinvade corn crops in cooler regions of North America, including Canada, during the summer [28]. Although fall armyworm can attack all corn stages, its injury is typically related to foliar consumption and indirect damage to grain production due to a reduction in the photosynthetic area [29]. Consequently, much *S. frugiperda* research in corn has been directed toward its management in early corn stages, generally when the whorl region is still present, where most fall armyworm larvae are found feeding on the developing leaves [30]. Although the fall armyworm has been reported to behave similarly to the corn earworm, *Helicoverpa zea* (Lepidoptera: Noctuidae), penetrating the ear and feeding on kernels a better understanding of fall armyworm infestation in reproductive corn stages is still necessary, as well as why the larvae "choose" that feeding site. Such behavior tends to inflict greater damage to corn because of injury to reproductive plant components. It could also limit the use of important control strategies, such as biological and conventional control through pesticide spraying because the larvae will be protected in the ear [31].

Insecticides are used widely as a tool in fall armyworm management both in the Americas [32] and in Africa [13]. Therefore, it is necessary to determine the field efficacy of insecticides on fall armyworm to integrate with Integrated Pest Management practices. The fall armyworm larva feeds by remaining most of its life in the whorl of maize, thus reducing its contact with insecticides. Multiple sprays of insecticides may lead to the quick development of resistance as has occurred in other areas [32]. Dose mortality response to insecticides is necessary to provide baseline data for future resistance monitoring [33]. Several newer insecticides have been developed in recent years having different modes of action for control of lepidopteran pests, to which the fall armyworm has yet to be exposed [13, 32, 34]. For decades, the main tool for fall armyworm control has been the use of synthetic insecticides [35]. After the commercialization of genetically engineered (GE) crops producing *Bacillus thuringiensis* insecticidal proteins in the United States, synthetic insecticide use decreased by 47.8% [36]. However, insecticide use remains high in countries where *Bt* technology is not available. As a result, fall armyworm is resistant to at least 29 insecticidal

active ingredients in six mode-of-action groups, in the Americas. Furthermore, fall armyworm has developed fieldevolved resistance to the Bt proteins Cry1F, Cry1Ac and Cry1Ab in Puerto Rico [35, 37].

Active ingredients with novel modes of action are available, including novaluron, flubendiamide, chlorantraniliprole, and cyantraniliprole. However, low corn prices coupled with high input costs often result in farmers choosing older, cheaper insecticides to save money. Resistance is a key issue in any cropping system where insecticides are heavily used and monitoring the susceptibility of a target pest is important for effective integrated pest management (IPM) and insecticide resistance management (IRM). Consumption and utilization parameters are important because they are needed to determine whether plant/chemical resistance affects insect behavior and/or metabolism [38]. Consumption and utilization indices also can be used as indicators of the Interaction between the insect and its food source treated or untreated [39]. The effects of insecticides on insects put uncommon symptoms of poisoning. Such symptoms indicated that the actual cause of death is either a rupture of the newly formed cuticle or interference with feeding, Nevertheless, information about the effect of insecticides on feeding and utilization of food is still meager [40].

The feeding or consumption rate of insects can be measured as the amount of foliage consumed on fresh weight, dry weight, or leaf area basis. Feeding rate is usually determined by measuring the reaction of the insect to food plant, and water content including the physical and chemical properties of food [41]. Consumption should be measured only on the later larval instars since early instars consume less food and it is difficult to quantify the amount consumed. The first three instars of FAW larvae consumed less than 27 of the total food intake, while the last three instar consumed 98% [8]. Utilization can be measured by three indices which include approximate digestibility (AD), the efficiency of conversion of Ingested food (ECI) or gross efficiency of growth, and efficiency of digested food (ECD) or net efficiency of growth. Utilization can be measured by gravimetric methods [41]. Keeping in view the aforementioned facts, the present research focuses on the assessment of the impact of insecticides on feeding behavior, food consumption and survival potential of *Spodoptera frugiperda* which has recently invaded Pakistan and is causing severe losses in maize crop.

II. MATERIALS AND METHODS

Insecticides

Formulated insecticides were used in all bioassays. These insecticides were Karate 5 EC (Lambda-cyhalothrin), Proclaim 5 SG (Emamectin benzoate), Syngenta, Pakistan, Delegate 11.7 SC (Spinetoram), Corteva Agrisciences Pakistan, Fame (Flubendiamide 48% SC), Bayer Crop Science, Pakistan, Coragen (Chlorantraniliprole 20% SC), Steward 150 EC (Indoxacarb) and Corvus 10 EC (Novaluron) FMC Pakistan, Pakistan were used for laboratory bioassay. Chlorantraniliprole (125 ml/ha), Spinetoram (200 ml/ha), Emmamectin benzoate (500 ml/ha), Indoxacarb (440 ml/ha), Lambda-cyhalothrin (620 ml/ha), Flubendiamide (60 ml/ha) and Novaluron (750 ml/ha) concentrations were used to observe super-lethal, lethal, median-lethal and sub-lethal effects.

Rearing of fall armyworm

Different growth stages of S. frugiperda were collected from fodder crops of maize which has not been exposed to any chemical or microbial treatments. All the collected stages were kept in separate plastic jars and brought to Integrated Pest Management (IPM) Laboratory, Department of Entomology, Sindh Agriculture University, Tandojam, Sindh, Pakistan. These collected larvae were reared on a natural diet until pupation. The pupae recovered were placed in special cages ($60 \times 60 \times 50$ cm) till adult emergence from pupae. An artificial moth diet of 10% honey solution soaked in cotton wool was placed in cages for feeding of moths. Leaves of maize plants were placed in the cages to facilitate the oviposition and replaced daily while the leaves having eggs were removed from the cage and shifted into separated ventilation cages $(30 \times 30 \times 40 \text{ cm})$ for the eggs to hatch. The hatching larvae were separated with attached leaves and put into clean cages ($60 \times 60 \times 50$ cm) having tissue paper to absorb the extra moisture and nylon mesh on both sides for ventilation. The artificial diet was supplied daily as a food until they pupate. The pupae of FAW were collected from cages using a camel hairbrush and transferred into cages ($60 \times 60 \times 50$ cm) for adult emergence. The adults will be shifted into separated cages $(30 \times 30 \times 40 \text{ cm})$ for oviposition and butter papers were used as egg receptacles instead of maize leaves. The butter paper was replaced after 24 hours to get the uniform age of eggs in a cylindrical plastic cage (20 cm dia., 15 cm height.) having a male and female ratio of 1:1 for oviposition. The top and bottom of each cage were modified having filter paper strips on the top side for oviposition while filter paper was glued with a plastic cover (20.5 cm dia.) on the bottom side. A homogeneous population of S. frugiperda was reared and maintained in Laboratory at 26±5°C, 51-70% RH and 12:12 L: D, photoperiod.

Experimental procedure and data collection

The first-generation population was used for the mortality bioassay analysis. Sublethal, median lethal (LC_{50}), lethal, and super-lethal concentrations for various insecticides were determined using a leaf-dip bio-assay system with minor modifications. For the monitor, a young, uniformly shaped maize-leaf disc (4 cm length x 3 cm width) from a 21-dayold seedling was submerged in an aqueous-insecticide solution for 12 seconds while a gentle agitation in distilled water for control. The treated-leaf discs were air-dried before being, placed in plastic cups (6 cm x 6 cm) and sealed. On each leaf bit, (20 larvae) of newly hatched second instar larvae were released for feeding. Each concentration was replicated three times. After 24 hours of exposure, the mortality rate was determined.

Second instar larvae were exposed to treated leaves and surviving larvae were transferred individually to plastic vials (5 cm in diameter by 6 cm in height) and applied with non-treated synthetic diets. Each vial was covered by a circular cardboard lid and kept upside-down to easily the collection of larval-excreted materials. Throughout the experiment, the total duration of the larval stage, food intake, larval weight gain, excrete development, and larval mortality was measured. The initial mass of each larva was notified quickly before the experiment was subtracted from the final body weight throughout the last larval instar to achieve the overall increase in single larval weight. At 48-hour intervals, the fresh and dry weight of the diet was reported before and after it is given to the larvae. Each larva feces were collected daily, dried to a constant mass in a hot air oven at 60°C, and weighed. Dry weight was used to analyze the nutrition information. Additional insects and nutrition cups were held under the same laboratory conditions to calculate the correction factors in both larvae and consumed food based on the fresh weight/dry weight proportion. The related ratio was multiplied by the larvae's fresh masses and the food consumed. If larvae do not move after being prodded, they were thought to be dead.

Data analysis

The collected data on mortality were subjected to probit analysis to determine sublethal, median-lethal, lethal and super-lethal concentrations of each insecticide. The data collected on various parameters of feeding behavior, food consumption and survival potential were subjected to the ANOVA technique for determining parameters of importance and significant means were compared by Tukey Honestly Significant Difference, (HSD) test on probability level by 5%.

III. RESULTS

Mortality of S. frugiperda first instar larvae

Results indicate that different insecticides, chlorantraniliprole, emmamectin benzoate, flubendiamide, spinetoram, lambda-cyhalothrin, indoxacarb and novaluron with super-lethal, lethal, median-lethal and sub-lethal concentrations had a significant effect on the 1^{st} instar larvae mortality (Table 1). Significant variations were observed by the application of different insecticides. It was overall observed that an increase in concentrations led to an increase in mortality but a decrease in concentrations led to a decrease in mortality. Irrespective of insecticides, chlorantraniliprole exhibited the maximum mortality (> 90%) at different doses and the novaluron exhibited minimum mortality (23%). Different concentrations are dependent on mortality because of the application of different insecticides.

Performance of chlorantraniliprole as superlethal, lethal, median-lethal and sub-lethal concentrations demonstrated that 100, 95, 90 and 86% mortality was observed and there was no mortality observed in the control treatment. Performance of emamectin benzoate as super-lethal, lethal, median-lethal and sub-lethal concentrations demonstrated that 91, 83, 71 and 60% mortality was observed and there was no mortality observed in the control treatment. Performance of flubendiamide with Super-lethal, lethal, median-lethal and sub-lethal concentrations demonstrated that 93, 86, 76 and 56% mortality was observed and there was no mortality observed in the control treatment. Performance of spinetoram in super-lethal, lethal, median-lethal and sub-lethal concentrations demonstrated that 91, 88, 73 and 50% mortality was observed and there was no mortality observed in the control treatment. Performance of indoxacarb as super-lethal, lethal, median-lethal and sub-lethal concentrations demonstrated that 73, 78, 70 and 46% mortality was observed and there was no mortality observed in the control treatment. Performance of lambda-cyhalothrin as super-lethal, lethal, median-lethal and sub-lethal concentrations demonstrated that 91, 81, 75 and 40% mortality was observed and there was no mortality observed in the control treatment. Irrespective of insecticides, novaluron exhibited the lowest mortality at different concentrations and it was observed that the concentrations dependent on mortality. Performance of indoxacarb as super-lethal, lethal, median-lethal and sub-lethal concentrations demonstrated that 73, 78, 70 and 46% mortality was observed and there was no mortality observed in the control treatment. Mean mortality of fall armyworm showed that the highest mortality (92.91%) was recorded at the field recommended dose of chlorantraniliprole with 4 different formulations (super-lethal, lethal, median-lethal and sublethal) while the least mortality (24.16%) was noticed by application of novaluron concentrations. Mortality of S. frugiperda was found dose-dependent and quick response to insecticide (Table 1).

Insect	Super-lethal	Lethal	Median- lethal	Sub-lethal	Mean±SE
Chlorantraniliprole	100.0±0.0	95.0±2.89	90.0±2.89	86.67±1.67	92.91±1.79
Emamectin benzoate	91.67±1.67	83.33±1.67	71.67±3.33	60 ± 2.89	76.67±3.76
Flubendiamide	93.33±4.41	86.67±1.67	76.67±1.67	56.67±3.33	78.33±4.37
Spinetoram	91.67±4.41	88.33±4.41	73.33±7.26	50 ± 2.89	75.83±5.39
Indoxacarb	73.33±6.67	70.33±3.33	56±5.77	46.67±4.41	67.08 ± 4.28
Lambda-cyhalothrin	91.67±6.01	81.67±6.01	75 ± 2.89	40 ± 7.64	72.08±6.38
Novaluron	35±5.77	28.33±4.41	20 ± 2.89	13.33±3.33	24.16±3.07
Control	0±0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0

Table 1. Comparison of mortality (%) of *Spodoptera frugiperda* at an altered dose rate of different insecticide formulations.

Mortality (%) of first instar larvae of S. frugiperda

Results indicate that different insecticides, chlorantraniliprole, emmamectin benzoate, flubendiamide, spinetoram, lambda-cyhalothrin, indoxacarb and novaluron with super-lethal, lethal, median-lethal and sub-lethal concentrations had a significant effect on the 1st instar larval mortality and food consumption P < 0.05. Irrespective of insecticides, chlorantraniliprole exhibited the maximum mortality (> 90%) at different doses and the novaluron exhibited minimum mortality (23%). Different concentrations are dependent on mortality because by application of different insecticides it was observed that an increase in concentrations led to an increase in mortality but a decrease in concentrations led to a decrease in mortality. Performance of chlorantraniliprole as super-lethal, lethal, median-lethal and sub-lethal concentrations demonstrated that 100, 95, 90 and 86% mortality was observed and there was no mortality observed in the control treatment. Performance of emamectin benzoate as super-lethal, lethal, median-lethal and sub-lethal concentrations demonstrated that 91, 83, 71 and 60% mortality was observed and there was no mortality observed in the control treatment. Performance of flubendiamide as super-lethal, lethal, median-lethal and sub-lethal concentrations demonstrated that 93, 86, 76 and 56% mortality was observed and there was no mortality observed in the control treatment. Performance of spinetoram in super-lethal, lethal, median-lethal and sub-lethal concentrations demonstrated that 91, 88, 73 and 50% mortality was observed and there was no mortality observed in the control treatment. Performance of indoxacarb as super-lethal, lethal, median-lethal and sub-lethal concentrations demonstrated that 73, 78, 70 and 46% mortality was observed and there was no mortality observed in the control treatment. Performance of lambda-cyhalothrin as super-lethal, lethal, median-lethal and sub-lethal concentrations demonstrated that 91, 81, 75 and 40% mortality was observed and there was no mortality observed in the control treatment. Irrespective of insecticides, novaluron exhibited the lowest mortality at different concentrations and it was observed that the concentrations dependent on mortality. Performance of Novaluron as super-lethal, lethal, medianlethal and sub-lethal concentrations demonstrated that 35, 28, 20 and 13% mortality was observed and there was no mortality observed in the control treatment (Table 2, 3, 4 and 5). The result indicates that insecticide formulations had a significant effect on the 2nd instar larval mortality, food consumption, weight gain, development time, the weight of ingested food (WIF), relative consumption Rate (RCR), final weight gain (FWG), relative growth rate (RGR), the weight of feces, approximate digestibility (AD), ingested food to body substance (ECI), the efficiency of conversion of digested food into growth (ECD) and assimilation rate (AR). These insecticides are used at the recommended field dose with super-lethal, lethal, median-lethal and sub-lethal concentrations to reduce the amount of survived larvae and food ingested by S. frugiperda larvae after treatment of the second instar.

Table 2. Effects of insecticides applied at the super lethal concentration on feeding, consumption, digestion, and growth of first instar larvae of *S. frugiperda* by nutritional indices.

Insecticides	Mortality (%)	Larval Survival	WIF (mg)	RCR	RGR	FWG (mg)	WEx	AD	ECI	ECD	AR
Chlorantraniliprole	100.0±0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Emamectin benzoate	91.67±1.67	1.67 ± 0.33	0.06 ± 0.03	0.53 ± 0.24	0.21±0.0	0.02 ± 0.0	0.0 ± 0.0	86.89 ± 10.11	104.62 ± 75.51	147.81 ± 17.79	50.61±23.82
Flubendiamide	93.33±4.41	1.33 ± 0.88	0.04 ± 0.04	0.39 ± 0.35	0.15 ± 0.08	0.02 ± 0.01	0.01 ± 0.01	51.39 ± 26.54	88.92 ± 78.03	103.06±86.53	26.36±22.82
Spinetoram	91.67±4.41	1.67 ± 0.88	0.03 ± 0.02	0.29 ± 0.20	0.15 ± 0.07	0.02 ± 0.01	0.01 ± 0.01	53.41 ± 27.42	45.96 ± 30.83	54.36 ± 33.35	21.82±13.77
Indoxacarb	73.33±6.67	5.33±1.33	0.13 ± 0.03	1.16 ± 0.38	0.25 ± 0.01	0.03±0.0	0.04 ± 0.02	74.60 ± 8.01	23.30 ± 4.02	31.42 ± 5.45	84.55±16.50
Lambda-cyhalothrin	91.67±6.01	1.67 ± 1.20	0.05 ± 0.05	0.43 ± 0.43	0.08 ± 0.08	0.01 ± 0.01	0.02 ± 0.02	22.22 ± 22.22	6.38 ± 6.38	9.57±9.57	28.48 ± 28.48
Novaluron	35.0 ± 5.77	$13.0{\pm}1.15$	0.72 ± 0.15	6.57±1.37	0.28 ± 0.01	0.03±0.0	0.25 ± 0.09	67.88 ± 5.20	4.71±0.89	6.82 ± 0.82	432.12 ± 60.74
Control	0.0 ± 0.0	20.0±0.0	1.15 ± 0.07	10.44 ± 0.66	0.38 ± 0.02	0.04 ± 0.0	0.44 ± 0.06	60.73 ± 8.06	3.71±0.40	6.56±1.73	644.85 ± 87.38

(RCR) Relative consumption rate, (RGR) Relative growth rate, (AD) Approximate digestibility, (ECI) Efficiency of conversion of ingested food to body substance, (ECD) Efficiency of conversion of digested food into growth, (AR) Assimilation rate, (WIF) Weight of ingested food, (WEx) Weight of excretion, (FWG) Final weight gain.

Super-lethal, sub-lethal, median lethal and lethal effects of insecticides

On a weight basis, food consumed was recorded for larvae fed for 24 h on chlorantraniliprole, spinetoram, emmamectin benzoate, lambda-cyhalothrin, flubendiamide and novaluron with super-lethal, lethal, median-lethal and sub-lethal doses of treated leaves as compared with control larvae. In all treatments, daily food consumption was inconsistent during the first 5 days, while a considerable increase was noticed after one week. A similar trend was also observed in the control larvae, though at a higher magnitude. Likewise, the consumption index decreased remarkably during the whole experimental period except after one week when remarkable increases were noticed indicating that the values of the consumption index were directly associated with the decrease recorded in the weight of consumed food. Higher concentrations of chlorantraniliprole, flubendiamide and emamectin benzoate had adverse effects on larval weight gain. This was most significant with super-lethal doses where weight gain was reduced relative to the control for the low and high dosages, respectively. Although the higher dosage of chlorantraniliprole, flubendiamide and emmamectin benzoate inhibited weight gain, the lower concentration seemingly stimulated weight gain. As the insecticide concentration increased, weight gain was reduced as compared to lower concentrations, respectively, relative to the control. Super-lethal doses led to significantly less larval weight gain relative to the control.

RGR and nutritional indices of larvae during the entire larval developmental period following the treatment with all insecticides except novaluron, at each applied concentration. After a short period of feeding on treated food, a decrease in the calculated values of the RGR (grams of tissue gained per gram of caterpillar per day) during the entire larval stage was observed; the magnitude of this impact was greater at higher insecticide concentration levels. Larvae exposed to low and high concentrations of synthetic insecticides exhibited a decrease in the RGR relative to that observed for the control group in the course of larval development. Similarly, chlorantraniliprole, flubendiamide and emmamectin benzoate caused a reduction in RGR of larvae fed diet treated with four concentrations but in the case of novaluron increase in RGR value. At the higher concentrations, all insecticides except novaluron caused statistically significant decreases in RCR value relative to the control. The consumption rate was not inhibited for either product when applied at low concentrations.

The post-ingestive influence of synthetic insecticides on the ability of larvae to convert ingested (ECI) food into their biomass was different for insecticides tested. The impact of chlorantraniliprole, flubendiamide and emmamectin benzoate was significantly greater than other insecticides at both high and low dosages. Decrease in the efficiency of conversion of ingested food for super-lethal and lethal doses. A similar trend was observed in the efficiency of the larvae to convert the digested food to growth (ECD); however, the effects of products on ECD were not statistically significant.

The percent of consumed food that is digested, which is described as AD, was moderately but significantly affected by most of the insecticide treatments. However, the lower concentration of insecticides did not affect the larvae significantly. AR was also affected by chlorantraniliprole, flubendiamide and emmamectin benzoate. On average, super-lethal doses exerted more inhibitory activity on the ARs of larvae than novaluron concentrations. The AR of larvae treated with insecticides was found to be reduced at the high concentration except in novaluron, relative to the control. Similarly, chlorantraniliprole greatly lessened the AR by when applied at a high concentration. However, there was not a statistically significant suppression of AR with the lower concentration.

Insecticides	Mortality (%)	Larval Survival	WIF (mg)	RCR	RGR	FWG (mg)	WEx	AD	ECI	ECD	AR
Chlorantraniliprole	$95.0{\pm}2.89$	1.0 ± 0.58	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	$0.0{\pm}0.0$	0.0 ± 0.0	0.0 ± 0.0
Emamectin benzoate	$83.33 {\pm} 1.67$	3.33 ± 0.33	0.12 ± 0.05	1.05 ± 0.41	$0.24{\pm}0.01$	0.03 ± 0.0	0.04 ± 0.01	68.14 ± 0.75	29.39 ± 9.56	43.15±13.93	72.12 ± 28.91
Flubendiamide	86.67 ± 1.67	2.67 ± 0.33	0.06 ± 0.02	$0.53{\pm}0.18$	0.23 ± 0.01	0.03 ± 0.0	0.02 ± 0.01	64.37 ± 4.78	66.06 ± 32.78	87.47 ± 46.25	$33.94{\pm}12.36$
Spinetoram	$88.33{\pm}4.41$	2.33 ± 0.88	0.08 ± 0.04	0.68 ± 0.36	0.15 ± 0.08	0.02 ± 0.01	0.02 ± 0.01	47.08 ± 23.54	15.14 ± 7.71	$21.45{\pm}10.94$	$48.18{\pm}25.43$
Indoxacarb	70.33±3.33	4.33±0.67	0.15 ± 0.04	$1.39{\pm}0.38$	$0.24{\pm}0.01$	0.03 ± 0.0	0.04 ± 0.01	70.16±4.09	$21.09{\pm}6.84$	31.21±11.67	82.61 ± 31.58
Lambda-cyhalothrin	$81.67{\pm}6.01$	3.67 ± 1.20	0.21 ± 0.15	$1.86{\pm}1.36$	$0.23{\pm}0.01$	0.03 ± 0.0	0.06 ± 0.04	69.06 ± 2.40	48.92 ± 33.26	73.11 ± 50.06	$91.15{\pm}62.39$
Novaluron	28.33 ± 4.41	14.33 ± 0.88	1.0 ± 0.0	9.06±0.03	0.31 ± 0.01	0.03 ± 0.0	0.32 ± 0.01	$67.58{\pm}1.03$	$3.38{\pm}1.10$	5.0 ± 0.09	95.42 ± 7.43
Control	0.0 ± 0.0	20.0±0.0	1.52 ± 0.06	13.78 ± 0.55	$0.39{\pm}0.01$	$0.04{\pm}0.0$	0.44 ± 0.01	70.77±1.50	2.86 ± 0.02	4.05 ± 0.12	$98.97{\pm}59.08$

Table 3. Effects of insecticides applied at the lethal concentration on feeding, consumption, digestion, and growth of *S. frugiperda* 2nd instar larvae by nutritional indices.

(RCR) Relative consumption rate, (RGR) Relative growth rate, (AD) Approximate digestibility, (ECI) Efficiency of conversion of ingested food to body substance, (ECD) Efficiency of conversion of digested food into growth, (AR) Assimilation rate, (WIF) Weight of ingested food, (WEx) Weight of excretion, (FWG) Final weight gain.

Table 4. Effects of insecticides applied at the median-lethal concentration on feeding, consumption, digestion, and growth of *S. frugiperda* second instar larvae by nutritional indices.

Insecticides	Mortality (%)	Larval Survival	WIF (mg)	RCR	RGR	FWG (mg)	WEx	AD	ECI	ECD	AR
Chlorantraniliprole	$90.0{\pm}2.89$	2.0 ± 0.58	0.03 ± 0.03	0.32 ± 0.28	0.14 ± 0.07	0.02 ± 0.01	0.01 ± 0.01	$45.80{\pm}22.96$	$117.43{\pm}78.79$	165.31±67.71	$20.91{\pm}18.68$
Emamectin benzoate	71.67±3.33	5.67±0.67	0.16 ± 0.03	1.47 ± 0.26	0.23 ± 0.0	0.03 ± 0.0	0.05 ± 0.01	67.61±2.89	16.50 ± 3.01	24.12±3.39	$97.88{\pm}13.14$
Flubendiamide	76.67±1.67	4.67±0.33	0.22 ± 0.05	2.02 ± 0.46	0.24 ± 0.0	0.03 ± 0.0	0.06 ± 0.03	76.44±9.51	$13.0{\pm}2.58$	16.79±1.96	$147.58{\pm}21.32$
Spinetoram	73.33±7.26	5.33±1.45	0.51 ± 0.25	4.59 ± 2.30	0.25 ± 0.02	0.03 ± 0.0	0.15 ± 0.08	$71.52{\pm}1.50$	9.37±4.56	13.12±6.49	$325.45{\pm}58.62$
Indoxacarb	$56.0{\pm}5.77$	$6.0{\pm}1.15$	0.55 ± 0.23	$4.99{\pm}2.09$	0.27 ± 0.02	0.03 ± 0.0	0.17 ± 0.07	$68.83{\pm}0.53$	6.78±1.73	$9.88 {\pm} 2.55$	344.55 ± 95.67
Lambda-cyhalothrin	$75.0{\pm}2.89$	5.0 ± 0.58	0.21 ± 0.02	1.95 ± 0.21	0.25 ± 0.02	0.03 ± 0.0	0.21 ± 0.14	14.48 ± 86.07	$13.40{\pm}2.37$	7.09 ± 8.44	6.06 ± 80.42
Novaluron	20.0±2.89	16.0±0.58	$0.74{\pm}0.15$	6.70±1.34	0.32±0.02	$0.04{\pm}0.0$	$0.23{\pm}0.06$	70.04±1.78	$5.20{\pm}1.14$	7.36±1.46	464.85±81.72
Control	0.0±0.0	20.0±0.0	0.95 ± 0.36	8.61±3.31	0.39±0.02	0.04 ± 0.0	0.20±0.13	82.39±8.66	5.68 ± 1.44	$6.85{\pm}1.68$	$678.79{\pm}89.05$

(RCR) Relative consumption rate, (RGR) Relative growth rate, (AD) approximate digestibility, (ECI) Efficiency of conversion of ingested food to body substance, (ECD) Efficiency of conversion of digested food into growth, (AR) Assimilation rate, (WIF) Weight of ingested food, (WEx) Weight of excretion, (FWG) Final weight gain.

Table 5. Effects of insecticides applied at the sub-lethal concentration on feeding, consumption, digestion, and growth of *S. frugiperda* larvae as judged by nutritional indices (mean values \pm SE).

Insecticides	Mortality (%)	Larval Survival	WIF (mg)	RCR	RGR	FWG (mg)	WEx	AD	ECI	ECD	AR
Chlorantraniliprole	$86.67{\pm}1.67$	2.67 ± 0.33	$0.04{\pm}0.02$	$0.39{\pm}0.21$	0.15 ± 0.07	0.02 ± 0.01	0.01 ± 0.01	$47.96{\pm}24.13$	$26.88{\pm}14.79$	$38.16{\pm}21.87$	$28.79{\pm}16.28$
Emamectin benzoate	$60.0{\pm}2.89$	8.0 ± 0.58	$0.27{\pm}0.07$	2.48 ± 0.63	$0.24{\pm}0.0$	0.03 ± 0.0	0.07 ± 0.01	72.75 ± 3.05	$10.94{\pm}2.32$	$15.30{\pm}3.59$	$183.64{\pm}53.64$
Flubendiamide	$56.67{\pm}3.33$	8.67 ± 0.67	$0.38{\pm}0.18$	$3.46{\pm}1.66$	0.25 ± 0.01	0.03 ± 0.0	0.17 ± 0.11	65.13±9.31	12.16 ± 5.55	$17.41{\pm}6.80$	$195.76{\pm}66.14$
Spinetoram	$50.0{\pm}2.89$	10.0 ± 0.58	$0.73{\pm}0.05$	6.63 ± 0.47	0.27 ± 0.01	0.03 ± 0.0	0.22 ± 0.01	69.77 ± 2.87	4.05 ± 0.29	5.85 ± 0.57	$465.15{\pm}51.55$
Indoxacarb	$46.67{\pm}4.41$	$10.67{\pm}0.88$	0.75 ± 0.17	$6.84{\pm}1.56$	0.27 ± 0.01	0.03 ± 0.0	0.21 ± 0.06	$72.62{\pm}1.63$	$4.55{\pm}1.34$	$6.20{\pm}1.72$	$492.12{\pm}80.53$
Lamdacyhalothrin	40.0 ± 7.64	$12.0{\pm}1.53$	$0.64{\pm}0.28$	5.85 ± 2.59	0.30 ± 0.03	0.03 ± 0.0	0.18 ± 0.08	71.16±1.04	$6.72{\pm}1.75$	$9.50{\pm}2.54$	$420.0{\pm}75.38$
Novaluron	13.33±3.33	17.33 ± 0.67	1.16 ± 0.31	$10.54{\pm}2.79$	0.35 ± 0.02	$0.04{\pm}0.0$	0.35 ± 0.09	69.69 ± 0.60	$3.97{\pm}1.24$	$5.72{\pm}1.84$	736.36 ± 85.38
Control	0.0±0.0	20.0±0.0	$1.39{\pm}0.40$	12.65±3.65	0.43 ± 0.01	0.05 ± 0.0	$0.39{\pm}0.14$	74.35±3.70	$4.48{\pm}1.85$	$5.84{\pm}2.12$	914.24±91.99

(RCR) Relative consumption rate, (RGR) Relative growth rate, (AD) Approximate digestibility, (ECI) Efficiency of conversion of ingested food to body substance, (ECD) Efficiency of conversion of digested food into growth, (AR) Assimilation rate, (WIF) Weight of ingested food, (WEx) Weight of excretion, (FWG) Final weight gain.

In the present research the LC_{20} , LC_{50} , LC_{80} and LC_{95} values for indoxacarb, lambda-cyhalothrin, and novaluron were not significantly different (95% confidence limit overlap), whereas the LC_{20} , LC_{50} , LC_{80} and LC_{95} for the remaining insecticides are significantly different from each another. The insecticides, chlorantraniliprole, emamectin benzoate and flubendiamide were the most toxic followed by indoxacarb, lambda-cyhalothrin, spinetoram and novaluron at different concentrations (Table 6).

Table 6. Field recommended dose (FRD) of insecticides.

Insecticides	FRD	$LC_{20}(x^2; P)$	$LC_{50}(x^2; P)$	$LC_{80}(x^2; P)$	$LC_{95}(x^2; P)$
<u>Chlennetter 219</u>	0.500/	0.0002	0.0027	0.0187	0.077
Chiorantraniiproie	0.50%	(2.39;0.302)	(2.39;0.302)	(2.39;0.302)	(2.39;0.302)
Emomentin honzoate	2 0%	0.0055	0.057	0.322	1.15
Emamectin benzoate	2.0%	(0.011;0.99)	(0.011;0.99)	(0.011;0.99)	(0.011;0.99)
Flubendiamide	0.25%	0.00092	0.0074	0.035	0.109
	0.23%	(1.309;0.502)	(1.309;0.502)	(1.309;0.502)	(1.309;0.502)
Spinotonom	0.80%	0.0053	0.033	0.127	0.344
Spinetorani		(3.11;0.21)	(3.11;0.21)	(3.11;0.21)	(3.11;0.21)
Indovacarh	0.18%	0.0017	0.057	0.777	5.31
Indoxacal b		(8.94;0.01)	(8.94;0.01)	(8.94;0.01)	(8.94;0.01)
I ambda avhalathrin	2 50%	0.0033	0.137	0.457	1.112
Lambua-cynaiothrin	2.3070	(7.23;0.027)	(7.23;0.027)	(7.23;0.027)	(7.23;0.027)
Novolunon	<u> 9 00/</u>	0.854	7.38	36.68	119.65
novaluron	8.0%	(0.22;0.89)	(0.22;0.89)	(0.22;0.89)	(0.22;0.89)

Field recommended doses of each insecticide with LC_{20} , LC_{50} , LC_{80} and LC_{95} values with chi-square and P values.

IV. Discussion

The sublethal concentrations of insecticides will affect the population dynamics of pests and have varying degrees of influence on each stage of insect growth and development. Sublethal effects were reported in several insect orders upon different biological, physiological, behavioral and demographic aspects, such as the effect of the aqueous extract of Trichilla sp. on the survival, development and larval and pupal weight of S. frugiperda [42]. In this study, the highest mortality was observed by chlorantraniliprole which is best for the management of this pest. Results are in support of Sharanabasappa [43] who investigated the control of second instar larvae of S. frugiperda by the leaf-dip bioassay method, as well as under field conditions both in June and September. Emamectin benzoate 5 SG showed the highest acute toxicity, followed by chlorantraniliprole18.5 SC, and spinetoram 11.7 SC, whereas toxicities of flubendiamide 480 SC, indoxacarb 14.5 SC, lambda-cyhalothrin5 EC, and novaluron10 EC were at par by the leafdip bioassay. The results of field efficacy for two planting dates (June sown crop, and September sown crop 2018) revealed that the Chlorantraniliprole, emamectin benzoate, and spinetoram were suitable as one of the components of Integrated Pest Management of fall armyworm in India. Results of Sisay [13] suggested that the nine synthetic insecticides belonging to different chemical groups and 11 pesticidal plants (botanicals) were tested for their efficacy against FAW under laboratory, greenhouse, and field conditions. In the laboratory, Radiant, Tracer, Karate, and Ampligo caused over 90% larval mortality 72 h after application. Malathion had moderate activity, causing 51.7% mortality 72 h after application, while Carbaryl was less effective, causing 28% mortality 72 h after application. In the greenhouse experiment, all synthetic insecticides reduced foliar damage to maize compared to the untreated control. In the field, non-treated control plants showed extensive leaf injury compared to the synthetic insecticide and botanical-treated plants. The synthetic insecticides and botanicals that showed high efficacy against FAW larvae can be used as components for integrated pest management (IPM) plans for FAW under smallholder farmer conditions in Ethiopia and elsewhere in Africa. S. frugiperda consumed less food (relative to control larvae) during the entire larval developmental period after having been fed for only one day during the second instar on diet treated with different insecticides. Feeding behavior is governed by both neural input from the insect's chemical senses (taste receptors on the tarsi and mouthparts) and central nervous integration of this sensory input. The primary antifeedant effects might be attributable to a direct action of synthetic insecticides, on the centers that control feeding and metabolism [44]. Conversion of ingested food by S. frugiperda larvae into biomass varied considerably among larvae fed synthetic insecticide formulations and four concentrations of each. The magnitude of suppression of food utilization was higher for chlorantraniliprole, emamectin benzoate and spinetorum. Overall, the growth of larvae, calculated as total weight gain and RGR, was inhibited by insecticides super-lethal except for novaluron, but the effect was dependent on the chemical formulation and concentration. Higher concentrations of these insecticide formulations caused lower RGRs and weight gain than lower concentrations. chlorantraniliprole, emamectin benzoate and spinetorum were more effective in the reduction of weight gain of larvae than other insecticides, even when applied at low concentrations. This indicates that the chemistry of these chemicals has a deleterious action on insect growth even after the feeding period has ceased, likely as a result of the reduction of food intake and the ability to convert food into biomass.

Toxicity studies showed that the LC₅₀ values of chlorantraniliprole, emamectin benzoate, and flubendiamide were found to be very low compared with, indoxacarb, lambda-cyhalothrin, spinetoram and novaluron. However, it is difficult to compare the susceptibility of our results to other studies given the different methods of bioassays to monitor susceptibility in fall armyworm. For instance, Gutiérrez-Moreno [32] studied the field-evolved resistance of the fall armyworm to different insecticides using topical applications and recorded the mortality 72 h post-treatment. The baseline of the susceptible population, for equivalent compounds other than the ones used in this research, indicated that the most toxic compound was emamectin benzoate followed by chlorantraniliprole, spinetoram, flubendiamide, triflumuron (a benzoylurea), and pyrethroids. In other residual bioassays, Belay [17] studied the effect of different insecticides on the management of fall armyworm larvae using a direct spray over third-instar larvae. More than 80% mortality was observed in chlorantraniliprole, flubendiamide, spinosad, indoxacarb, and fenvalerate treatments 96 h after application. Yet another study by overlay diet assay showed reduced LC50 values including chlorfenapyr (1.2 ppm), emamectin benzoate (0.0029 ppm), fipronil (2.4 ppm), and tebufenozide (0.95 ppm) [45]. Results of Shannag [46] showed that in diet-incorporated assays, the LC50 values of chlorantraniliprole (0.068 μ g mL⁻¹) and spinetoram (0.066 μ g mL-1) were significantly lower than the LC₅₀ of indoxacarb (0.392 μ g mL⁻¹) and flubendiamide (0.930 μ g mL⁻¹). Despite that the method of bioassay used by Hardke [26] was different from our method, the active ingredients for spinetoram and chlorantraniliprole exhibited similar levels of toxicity. Higher values of the slopes and (LC₉₅) were found with indoxacarb and lambda-cyhalothrin in our results. These results represent initial efforts to develop baseline susceptibility data for the insecticides that are currently used for the control of fall armyworm. These toxicity values help in monitoring changes in susceptibility to these new insecticides as their use becomes widespread on maize in the southern states of India. However, a standard bioassay method for fall armyworm is important to assess the susceptibility.

In the present research the LC_{20} , LC_{50} , LC_{80} and LC_{95} values for indoxacarb, lambda-cyhalothrin, and novaluron were not significantly different (95% confidence limit overlap), whereas the LC_{20} , LC_{50} , LC_{80} and LC_{95} for the remaining insecticides are significantly different from each another. The insecticides, chlorantraniliprole,

emamectin benzoate and flubendiamide were the most toxic followed by indoxacarb, lambda-cyhalothrin, spinetoram and novaluron at different concentrations.

The mean dry weight of food intake by larvae treated with different concentrations of insecticides was affected at higher concentrations of chlorantraniliprole, flubendiamide and emmamectin benzoate and the total ingested food by larvae relative to the control. However, the lower concentrations of all insecticides did not significantly affect the total amount of ingested food except chlorantraniliprole. Daily food consumption was inconsistent during the first 5 days, while a considerable increase was noticed after one week. A similar trend was also observed in the control larvae, though at a higher magnitude. Likewise, the consumption index decreased remarkably during the whole experimental period except after one week when remarkable increases were noticed indicating that the values of the consumption index were directly associated with the decrease recorded in the weight of consumed food. Higher concentrations of chlorantraniliprole, flubendiamide and emmamectin benzoate had adverse effects on larval weight gain. This was most significant with super-lethal doses where weight gain was reduced relative to the control for the low and high dosages, respectively. Although the higher dosage of chlorantraniliprole, flubendiamide and emmamectin benzoate inhibited weight gain, the lower concentration seemingly stimulated weight gain. As the insecticide concentration increased, weight gain was reduced as compared to lower concentrations, respectively, relative to the control. Super-lethal doses led to significantly less larval weight gain relative to the control. After a short period of feeding on treated food, a decrease in the calculated values of the RGR (grams of tissue gained per gram of caterpillar per day) during the entire larval stage was observed; the magnitude of this impact was greater at higher insecticide concentration levels. Larvae exposed to low and high concentrations of synthetic insecticides exhibited a decrease in the RGR relative to that observed for the control group during larval development. Similarly, chlorantraniliprole, flubendiamide and emmamectin benzoate caused a reduction in RGR of larvae fed diet treated with four concentrations but in the case of novaluron increase in RGR. At the higher concentrations, all insecticides except novaluron caused statistically significant decreases in RCR value relative to the control. The consumption rate was not inhibited for either product when applied at low concentrations. The post-ingestive influence of synthetic insecticides on the ability of larvae to convert ingested (ECI) food into their biomass was different for tested insecticides. The impact of chlorantraniliprole, flubendiamide and emmamectin benzoate was significantly greater than other insecticides at both high and low dosages. Decrease in the efficiency of conversion of ingested food for super-lethal and lethal doses. A similar trend was observed in the efficiency of the larvae [47] to convert the digested food to growth (ECD). The percent of consumed food that is digested, which is described as AD, was moderately but significantly affected by most of the insecticide treatments. However, the lower concentration of insecticides did not affect the larvae significantly. AR was also affected by chlorantraniliprole, flubendiamide and emmamectin benzoate. On average, super-lethal doses exerted more inhibitory activity on the ARs of larvae than novaluron concentrations. The AR of larvae treated with insecticides was found to be reduced at the high concentration except in novaluron, relative to the control. Similarly, chlorantraniliprole greatly lessened the AR by when applied at a high concentration. However, there was not a statistically significant suppression of AR with the lower concentration.

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