



Response of fall armyworm, *Spodoptera frugiperda* (J. E. Smith) to different pheromone blends under Indian environmental conditions

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ABSTRACT: Fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) is a pest of maize native to America that invaded India recently in 2018. Field and laboratory experiments were conducted in Bengaluru, India to find a suitable pheromone blend for trapping males of fall armyworm. The field evaluation studies showed that pheromone blend with Z-9-tetradecenyl acetate, Z-11-hexadecenyl acetate and Z-7-dodecenyl acetate in 87: 12.5 : 0.5 ratio attracted and trapped statistically significant number of male moths compared to other five blends using funnel traps. The laboratory evaluation of 6 different pheromone blends using Electroantennogram (EAG) with male antenna of *S. frugiperda* elicited statistically on par responses with each other. From the different dosage field study with optimum attracting blend, it was found that 2 mg loading trapped highest number of moths and was statistically on par with 3 mg pheromone lure. Further monitoring study carried out under farmer's field condition with 2 mg loading of pheromone blend with Z-9-tetradecenyl acetate, Z-11-hexadecenyl acetate and Z-7-dodecenyl acetate in 87: 12.5: 0.5 ratio found to be more effective in attracting and trapping male moths up to 60 days.

Keywords: Fall armyworm, *Spodoptera frugiperda*, pheromone, Z-9-tetradecenyl acetate, Z-11-hexadecenyl acetate, Z-7-dodecenyl acetate, evaluation

INTRODUCTION

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith), a destructive invasive pest of maize and several other grass crops such as sugarcane, sorghum, millets and vegetables is native to America which has spread to Africa in 2016 and later to Indian Subcontinent in 2018. In India, the occurrence of fall armyworm was first reported from Karnataka but soon spread to others states too (Sharanabasappa *et al.*, 2018a; Ganiger, 2018; Padhee and Prasanna, 2019; Ankush Chormule *et al.*, 2019 and Mallapur, *et al.*, 2018). The life cycle of fall armyworm, *S. frugiperda* (FAW) is completed 32-46 days. The female moth lays eggs under or on maize leaves surface, near base of plant and also in whorls. It has 6 larval instars and pupates in soil for 9-12 days. The adult are nocturnal, live for average 10 days. This invasive pest is voracious feeder and larval growth stage of 4-6 days old does the most damage by feeding foliage, protective leaf bracts into the side of cob, developing kernels (seeds) and feeding on young plants resulting in no leaves or cobs (Padhee and Prasanna, 2019; Sharanabasappa *et al.*, 2018b and CABI, 2016).

The sex pheromone of FAW was identified first in North America, from female abdominal tips as Z-9-tetradecenyl acetate (Sekul and Sparks, 1967) and later Z-9-dodecenyl acetate was identified from same source (A. A. Sekul and A. N. Sparks, 1976 and Richard L. Jones and Alton N. Sparks, 1979). Later additional compounds

such as Z-9-dodecenyl acetate, Z-7-dodecenyl acetate and Z-11-hexadecenyl acetate were reported which are critical for attraction in field (Tumlinson *et al.*, 1986). Also E-7-dodecenyl acetate found in female abdominal extract of Brazilian population was reported as active component in the sex pheromone of FAW (Mitchell *et al.*, 1985 and Luciane *et al.*, 2006). In Brazilian FAW population, the ternary component pheromone blend with Z-7-dodecenyl acetate, Z-9-dodecenyl acetate and E-7-dodecenyl acetate was found to be highly attractive in field (Luciane *et al.*, 2006). While in Cost Rica (Central America) the ternary pheromone component blend with Z-9-tetradecenyl acetate, Z-11-hexadecenyl acetate and Z-7-dodecenyl acetate was found to be effective in field trap captures of FAW (Romano *et al.*, 2000). In the present study, based on the previous knowledge of already identified pheromone components from various countries were tested with different blend combinations with minor modifications (mixture of 2, 3, 4 synthetic chemical/ compounds) in Bengaluru to investigate both at laboratory and field level to screen out the optimum blend for trapping FAW males in the field under Indian environmental conditions.

MATERIALS AND METHODS

Insects

Insects were collected from infested field in Chikka Tumkur, near Rajankunte, Bengaluru Rural (13.2563°N,

77.5204°E) and were examined at Bio-Control Research Laboratories (BCRL) and confirmed as *S. frugiperda* through morphological keys (Pogue, 2002; EPPO, 2015; Ganiger, 2018 and Sharanabasappa *et al.*, 2018a&b). The male moths were used for Electroantennogram laboratory study.

Pheromone Chemicals

The commercially available Z-9-tetradecenyl acetate, Z-9-dodecenyl acetate, Z-11-hexadecenyl acetate, Z-7-dodecenyl acetate and E-7-dodecenyl acetate were supplied by Gaiagen Technologies, Bengaluru for using in formulations for different studies. The isomeric purities of these chemicals are 99% based on analysis using Shimadzu Gas Chromatograph GC-2014 at BCRL, using a Lab SH-Stabilwax column (30 m length × 0.25 mm internal diameter × 0.50 µm film thickness, Shimadzu Asia Pvt Ltd, Singapore) fitted with flame ionization detector (FID). The column oven was programmed after an initial delay of 2 min from 60 °C to 220 °C at 6 °C per minute. Nitrogen gas was used as a carrier with a flow rate of 1.5 mL/min. One microliter of the sample was introduced in the split ratio of 10:1 at an injection temperature of 225 °C.

Laboratory study using EAG for comparing different pheromone blends

Electroantennogram is a technique which records the small voltage fluctuations between the tip and base of an insect antenna when subjected to stimulus. EAG was carried out with field collected male moths of *S. frugiperda*. The insect antenna was carefully excised and placed on EAG combiprobe antenna holder such that the base of the antenna is fixed towards +ve and tip of the antenna towards -ve. The Pasteur pipette with filter paper impregnated with 20µL of 2% synthetic test chemical and was used as stimulus to puff over the *S. frugiperda* male antenna using Syntech Stimulus controller CS-55 (with built in air pumps, activated carbon inlet filter to deliver purified continuous air flow). The antennal response elicited to the stimulus was recorded Table 1 using Syntech IDAC-2 (Intelligent Data Acquisition Controller) with software (EAGpro) controlled signal acquisition with built-in amplifier. The Insect antennal response in millivolts to different pheromone blends were statistically analyzed using one way ANOVA by DMRT method (IBM SPSS Statistics Software Version 25).

Comparison of five different pheromone blends (Field trial 1)

Field study was conducted in a farmer's field to compare five different pheromone blend combinations

as mentioned in Table 2. All the lures were prepared at BCRL Bengaluru, in white vials with 2 mg pheromone loading. The trial was carried out in FAW infested maize field (crop age of 35 days) in Chikka Tumkur, near Rajankunte, Bengaluru (13.2563°N, 77.5204°E) for one month (From 30th Aug to 29th Sep 2018) using four funnel traps per treatment installed in two acres field with trap placed at a height of 4 feet above the ground surface. The observations of number of moths trapped in different treatments were recorded regularly during the trial period. The results of number of moths trapped in different treatments were statistically analyzed using one way ANOVA by DMRT method (IBM SPSS Statistics Software Version 25).

Comparative field study between two different blends and their different dosages (Field Trial 2)

This field study carried out under farmer field condition was comparison between the best performing blend among five different blends from field trial No. 1 and 6th blend combination, each treatment with 2 mg and 3 mg loadings of pheromone quantity as mentioned in Table 3. The trial was carried out in FAW infested maize field (crop age of 35 days) in Arakere, near Rajankunte, Bengaluru (13.2000°N, 77.5381°E) for 50 days (from 18th Dec 2018 to 6th Feb 2019) using 5 Funnel traps per treatment installed in total 1.5 acres field with trap placed at a height of 4 feet above the ground surface. The observation of number of moths trapped in different treatments was recorded regularly during the trial period. The results of number of moths trapped in different treatment were statistically analyzed using one way ANOVA by DMRT method (IBM SPSS Statistics Software Version 25)

Monitoring of fall armyworm using pheromone traps

To monitor the adult populations of *S. frugiperda*, 5 funnel trap with lures were placed with 2 mg loading of pheromone blend combination of Z-9-tetradecenyl acetate, Z-11-hexadecenyl acetate and Z-7-dodecenyl acetate in 87 : 12.5 : 0.5 ratio in 1.5 acres maize crop at Chikka Tumkur, near Rajankunte, Bengaluru (13.2563°N, 77.5204°E). The fall armyworm infestation level was checked randomly selecting 100 plants and took count of healthy plant and damaged plant by visible symptoms and also taking count of number of larvae present. The trial was carried out from 20 days old crop and continued up to 2 months. Observation on number of moths caught in the trap and damage symptoms of plant were recorded.

Table 1. EAG response of fall armyworm, *Spodoptera frugiperda* male antenna to different pheromone blend combinations

Treatment No.	Treatment Details / Particulars	EAG Response in mV
T1	Z-9-Tetradecenyl acetate : Z-7-Dodecenyl acetate (100 : 0.5)	1.05 ± 0.172 ^a
T2	Z-9-Tetradecenyl acetate : Z-7-Dodecenyl acetate (100 : 2)	1.25 ± 0.193 ^a
T3	Z-9-Tetradecenyl acetate : Z-7-Dodecenyl acetate : Z-11-Hexadecenyl acetate (100 : 2 : 8)	1.21 ± 0.123 ^a
T4	Z-9-Tetradecenyl acetate : Z-7-Dodecenyl acetate : Z-11-Hexadecenyl acetate : Z-9-Dodecenyl acetate (100 : 2 : 8 : 0.25)	1.15 ± 0.115 ^a
T5	Z-9-Tetradecenyl acetate : Z-7-Dodecenyl acetate : E-7-Dodecenyl acetate (100 : 1 : 1)	1.21 ± 0.112 ^a
T6	Z-9-Tetradecenyl acetate : Z-11-Hexadecenyl acetate : Z-7-Dodecenyl acetate (87 : 12.5 : 0.5)	1.36 ± 0.202 ^a
T7	Control (Hexane)	0.84 ± 0.275 ^a
F Value		0.875
p Value		0.525

The mean difference is non-significant at alpha 0.05 level. Values followed by same letters are not significantly different at the 0.05 level by DMRT, IBM SPSS Software 25v.

RESULTS

Laboratory study using EAG for comparing different pheromone blends

The EAG comparative study was carried out with six different pheromone blend combinations against *S. frugiperda* male antenna. Male antenna responded to pheromone blend combination of Z-9-tetradecenyl acetate : Z-11-tetradecenyl acetate : Z-7-dodecenyl acetate in 87 : 12.5 : 0.5 ratio with 1.36 mV which is higher response compared to pheromone blend combination of Z-9-tetradecenyl acetate and Z-7-dodecenyl acetate in 100 : 2 ratio with 1.25 mV, followed by Z-9-tetradecenyl acetate, Z-7-dodecenyl acetate and E-7-dodecenyl acetate in 100 : 1 : 1 ratio with 1.21 mV, followed by Z-9-tetradecenyl acetate, Z-7-dodecenyl acetate and Z-11-hexadecenyl acetate in 100 : 2 : 8 ratio with 1.21 mV, followed by

Z-9-tetradecenyl acetate, Z-7-dodecenyl acetate, Z-11-hexadecenyl acetate and Z-9-dodecenyl acetate in 100 : 2 : 8 : 0.25 with 1.15 mV, followed by Z-9-tetradecenyl acetate : Z-7-dodecenyl acetate in 100 : 0.5 ratio with 1.05 mV and least response to control (Solvent hexane) with 0.84 mV (Table 1). The Statistical analysis by one way ANOVA using DMRT method showed statistically non-significant results at alpha 0.05 level. The EAG responses among different pheromone blend combinations were on par with each other.

Field trial 1 : Comparison of 5 different pheromone blends

The comparative study was carried out to find out the best blend among 5 different pheromone blends in attracting and trapping more number of *S. frugiperda*. In the comparative study, treatment T5 attracted and

trapped total 124 no. of *S. frugiperda* male moths having pheromone blend combination of Z-9-tetradecenyl acetate, Z-7-dodecenyl acetate and E-7-dodecenyl acetate in 100 : 1 : 1 ratio which is highest number of trapped moths, followed by treatment T4 with total 85 moths having pheromone blend combination of Z-9-tetradecenyl acetate, Z-7-dodecenyl acetate, Z-11-hexadecenyl acetate and Z-9-dodecenyl acetate in 100 : 2 : 8 : 0.25; followed by treatment T3 with total 65 moths having pheromone blend combination of Z-9-tetradecenyl acetate, Z-7-dodecenyl acetate and Z-11-hexadecenyl acetate in 100 : 2 : 8 ratio, followed by Treatment T2 with total 65 moths having pheromone blend combination of Z-9-tetradecenyl acetate and Z-7-dodecenyl acetate in 100 : 2 ratio and least trapped in treatment T1 with total 46 moths having pheromone blend combination of Z-9-tetradecenyl acetate : Z-7-dodecenyl acetate in 100 : 0.5 ratio. The statistical analysis by one way ANOVA using DMRT method showed non-significant results at alpha 0.05 level. Overall T5 showed highest number of catches and T1 showed the least number of catches and T2, T3, T4 gave on par with each other (Table 2). Hence the T5 having pheromone blend combination of Z-9-tetradecenyl acetate, Z-7-dodecenyl acetate and E-7-dodecenyl acetate in 100: 1 : 1 ratio was considered for comparative study in the second field trial with the best EAG responding blend.

Comparative field study between two blends with their different dosages (Field trial 2)

The comparative study was carried out between treatment Z-9-tetradecenyl acetate, Z-11-hexadecenyl acetate and Z-7-dodecenyl acetate in 87 : 12.5 : 0.5 ratio (highest in EAG study) and Z-9-tetradecenyl acetate : Z-7-dodecenyl acetate : E-7-dodecenyl acetate in 100 : 1 : 1 ratio (highest in field trial 1) with each 2 mg

and 3 mg loading to screen out best blend and dosage attracting and trapping highest number of *S. frugiperda* moths. From the field results it is found that pheromone blend combination with Z-9-tetradecenyl acetate, Z-11-hexadecenyl acetate and Z-7-dodecenyl acetate (87: 12.5: 0.5) attracted and trapped highest number of moths compared to Z-9-tetradecenyl acetate: Z-7-dodecenyl acetate: E-7-dodecenyl acetate (100: 1: 1) with statistical significance. From the dosage it is observed that in both the treatments of 2 mg and 3 mg loading statistically on par with each other (Table 4). However treatment with Z-9-tetradecenyl acetate, Z-11-hexadecenyl acetate and Z-7-dodecenyl acetate (87 : 12.5 : 0.5) with 2 mg loading trapped total 202 moths compared to 3 mg loading with 191 moths while treatment with Z-9-tetradecenyl acetate : Z-7-dodecenyl acetate : E-7-dodecenyl acetate (100 : 1 : 1) 3 mg loading trapped total 36 moths compared to 2 mg loading with 35 moths. The statistical analysis by one way ANOVA using DMRT method showed statistically significant results at alpha 0.05 level.

Monitoring of fall armyworm using pheromone traps

The monitoring study was carried out with 2mg loading of optimum responding blend Z-9-tetradecenyl acetate, Z-11-hexadecenyl acetate and Z-7-dodecenyl acetate (87 : 12.5 : 0.5) for 2 months with five pheromone lures and funnel traps. Even though the field was sprayed with regular pesticides as per the farmer's schedule, moth catches were observed in the traps indicating that the field contains the infestation and having resistance to pesticides. The total numbers of moths trapped were 251 in 9 weeks, with 27.8 per week, 3.98 per day and 0.80 moths per trap. The monitoring of infestation level was done by selecting 100 plants randomly and taking count of number of larva present per plant and number of

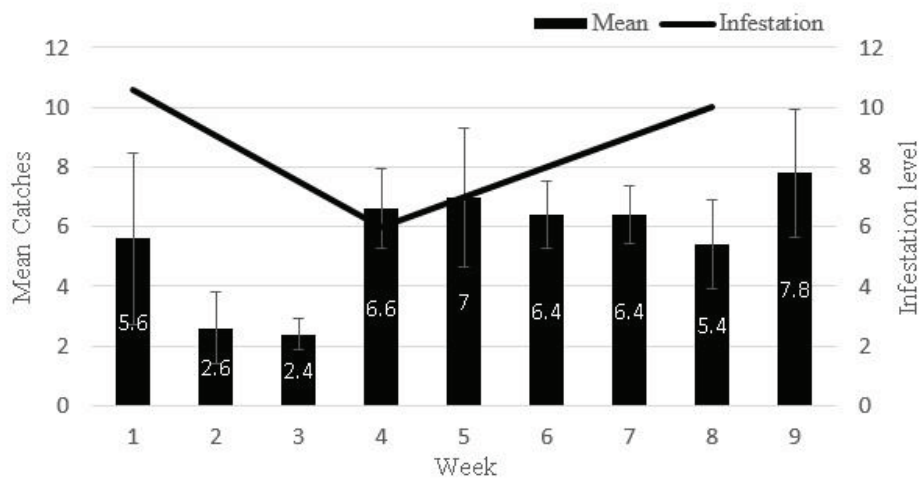


Fig.1. Monitoring of FAW infestation and field catches of adult moths using pheromone traps at Chikka Tumkur near Bengaluru

Table 2. Mean catches of fall armyworm, *Spodoptera frugiperda* to different pheromone blend combination lures with funnel traps in Chikka Tumkur field

Treatment No.	Treatments Details / Particulars	Total Catches	Mean Catches per trap/day \pm SEM
T1	Z-9-Tetradecenyl acetate : Z-7-Dodecenyl acetate (100 : 0.5)	46	1.92 \pm 0.752 ^a
T2	Z-9-Tetradecenyl acetate : Z-7-Dodecenyl acetate (100 : 2)	65	2.71 \pm 0.877 ^a
T3	Z-9-Tetradecenyl acetate : Z-7-Dodecenyl acetate : Z-11-Hexadecenyl acetate (100 : 2 : 8)	65	2.71 \pm 0.879 ^a
T4	Z-9-Tetradecenyl acetate : Z-7-Dodecenyl acetate : Z-11-Hexadecenyl acetate : Z-9-Dodecenyl acetate (100 : 2 : 8 : 0.25)	85	3.54 \pm 1.041 ^a
T5	Z-9-Tetradecenyl acetate : Z-7-Dodecenyl acetate : E-7-Dodecenyl acetate (100 : 1 : 1)	124	5.17 \pm 2.004 ^a
F Value			1.060
p Value			0.379

The mean difference is non significant at the 0.05 level. Values followed by same letters are not significantly different at the 0.05 level by DMRT, IBM SPSS Software 25v.

healthy and damaged plant (Fig. 1). From the monitoring studies it was observed that lures are effective in trapping FAW moths up to 60 days.

DISCUSSION

The fall armyworm, *S. frugiperda* outbreak reported in Karnataka around mid-May 2018 and later at various other states of India. An immediate action was required to manage the economic and yield loss to maize and other crops. In this regard, efforts were made towards to control FAW moths multiplication by one of the IPM strategy using bio-control method through pheromone lures and trapping system. The FAW reported in Africa and India in 2016 and 2018 are native to America, and their different sex pheromone components were already identified and their ratios were reported.

The first sex pheromone component was isolated and identified in North America was Z-9-tetradecenyl acetate from female abdominal gland which elicited wing fanning and copulatory behaviour in laboratory but failed to attract moths in the field (Sekul and Sparks, 1967). Later Z-9-dodecenyl acetate was identified from same source and its attractiveness was confirmed in

field (Sekul and Sparks, 1976). Further studies carried out gave significant result with binary mixture of these two components than its individual part (Richard L. Jones and Alton N. Sparks, 1979). Including this two pheromone components, additional compounds were identified from calling female FAW moths as Z-7-dodecenyl acetate and Z-11-hexadecenyl acetate which were critical for effectiveness of lure in the field. The four component pheromone blend which attracted more number of FAW moths contained (percentage by weight) Z-7-dodecenyl acetate (0.45%), Z-9-dodecenyl acetate (0.25%), Z-9-Tetradecenyl acetate (81.61%) and Z-11-hexadecenyl acetate (17.69%) with field viability of 2 weeks (Tumlinson *et al.*, 1986).

The pheromone lures from North America was not found effective in Brazil (South America) and Cost Rica (Central America). In the Brazilian *S. frugiperda* population, an active new component E-7-dodecenyl acetate was reported for the first time and found effective along with Z-7-dodecenyl acetate and Z-9-tetradecenyl acetate; pheromone identified in North America also present in Brazilian population with 0.01 : 0.01 : 100 ratio (Luciane *et al.*, 2006).

Table 3. Mean catches of fall armyworm, *Spodoptera frugiperda* to different pheromone blend with different loading in Arakere field

Treatment No.	Treatment Details / Particulars	Total Catches	Mean catches per trap/day \pm SEM
T1	2mg loading of Z-9-Tetradecenyl acetate : Z-7-Dodecenyl acetate : E-7-Dodecenyl acetate (100 : 1 : 1)	35	1.00 \pm 0.307 ^b
T2	3mg loading of Z-9-Tetradecenyl acetate : Z-7-Dodecenyl acetate : E-7-Dodecenyl acetate (100 : 1 : 1)	36	1.03 \pm 0.280 ^b
T3	2mg loading of Z-9-Tetradecenyl acetate, Z-11-Hexadecenyl acetate and Z-7-Dodecenyl acetate (87 : 12.5 : 0.5)	202	5.77 \pm 1.515 ^a
T4	3mg loading of Z-9-Tetradecenyl acetate, Z-11-Hexadecenyl acetate and Z-7-Dodecenyl acetate (87 : 12.5 : 0.5)	191	5.46 \pm 1.194 ^a
F Value			7.266
p Value			0.000***

The mean difference is significant at the 0.05 level. Values followed by same letters are not significantly different at the 0.05 level by DMRT, IBM SPSS Software 25v.

The Central America re-investigated the four acetate attractants (Z9-14Ac, Z11-16Ac, Z7-12Ac and Z9-12Ac) for high performance lure as North American lure gave inconsistent capture rates. The reinvestigation studies resulted in ternary blends of Z-9-tetradecenyl acetate, Z-11-hexadecenyl acetate and Z-7-dodecenyl acetate in the ratio of 87: 12.5: 0.5 significantly attractive in trapping FAW moths. This same pheromone blend ratio also found effective in Brazil (Romano *et al.*, 2000). But pheromone blend which found successfully attractive in North America was not effective in Central or South America, hence the geographical variations were observed in attractiveness in the field. Several studies have reported the intraspecific geographic variation in pheromone compositions of insects under selection pressures (Romano *et al.*, 2000; Bill S. Hansson *et al.*, 1990; Anglade *et al.*, 1984; Christer Lofstedt C *et al.*, 1986; Christer Lofstedt, 1990 and Miklos Toth *et al.*, 1992).

From our studies it was found that the binary and quaternary mixtures with slight modification in the blend ratio failed to attract high number of FAW moths. While the Brazilian pheromone blend combination with slight modification in ratio of Z-9-tetradecenyl acetate : Z-7-dodecenyl acetate : E-7-dodecenyl acetate as 100 : 1 : 1 was attractive than binary and quaternary mixture. While the pheromone blend Z-9-tetradecenyl acetate, Z-11-hexadecenyl acetate and Z-7-dodecenyl acetate in

the ratio of 87 : 12.5 : 0.5 which was found effective in Central America is also found to be effective in our studies carried out in Bengaluru (Karnataka State, India) by trapping significantly highest number of FAW moths. During FAW monitoring study using pheromone traps, it was observed that the three component blend is highly effective in attracting and trapping FAW moths in the field which is subjected to pesticide sprays.

CONCLUSION

From the above studies it is evident that fall armyworm, *S. frugiperda* responded well both in field and EAG studies to treatment having pheromone blend combination Z-9-tetradecenyl acetate, Z-11-hexadecenyl acetate and Z-7-dodecenyl acetate (87 : 12.5 : 0.5 ratio) attracted & trapped highest number of moths and highest EAG response in mV. From the dosage and monitoring studies it is concluded that pheromone lure loaded with 2 mg placed in funnel trap attracted and trapped highest number of FAW moths and recommended for monitoring purpose up to 2 months field validity. Hence the pheromone lure having ternary pheromone blend with Z-9-tetradecenyl acetate, Z-11-hexadecenyl acetate and Z-7-dodecenyl acetate (87:12.5:0.5) with 2 mg dosage is suitable and recommended for monitoring purpose in India. Further investigation needs to be done with the available FAW species for accurate details of their pheromone.

ACKNOWLEDGMENT

We would like to thank Mr. Subramanya, Field Officer, BCRL for carrying out the field trials as per requirement.

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MS Recieved - 29 May 2020

MS Accepted -11 June 2020