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Compatibility of Novel Chemical Compounds Against *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) in Field and Laboratory Condition

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Abstract: The present study has been carried out to record the performance of different pesticides against Helicoverpa armigera which is to be considered as one of the most destructive insect pests of chickpea. The field experiment was conducted at Pulse Section, ARI, Tandojam during the Rabi season 2017-18. Four different pesticides i.e. Emamectin benzoate 2.1EC, Indoxacarb 16.5EC, Flubendiamide 39.4SC and Emamectin benzoate + Lufenuron 2.7SC were applied. The results showed that after application of variable sprays, a significant reduction in pest population was observed at different intervals in field condition. The overall reduction percentage in pest population was the highest when Emamectin benzoate sprayed 2.1EC (63.92% at 24hrs, 62.03% at 48hrs, 60.00% at 72hrs and 58.90% at 1week) followed by Indox a carb. However, when Emamectin benzoate was added with Lufenuron 2.7SC, the results were not satisfactory. In, addition, Flubendiamide 39.4SC was also effective against pest but slow in action. Similarly, in laboratory bioassay, the best LT_{50} (17.72 hrs) and LT_{90} (51.25 hrs) were also observed at Proclaim at 50 ppm which further confirmed that this pesticide need short time to kill 50-90% targeted pest population. Therefore, Emamectin benzoate is highly suggested against H. armigera to reduce its population below economic threshold level.

Keywords: Helicoverpa Armigera, Pesticides, Mortality and Chickpea

1. INTRODUCTION

Gram pod borer, *Helicoverpa armigera* (Hubner) is one of the most destructive insect pests of chickpea. It damages the chickpea plants from seedling stage to crop maturity stage. All larval instars are responsible for damaging leaves, tender twigs, flowers and pods. However, severe damage occurs at pod formation when larvae bore inside the pods and cause significant yield loss. The pest mainly is controlled by application of distinct pesticides. About 30% of total world insecticides are reported to use against *H. armigera*; therefore, it is under high selection pressure in order to choose right pesticide against this pest (Ahmad, 2007). The activity of insect biotypes against several pesticides in different cropping systems worldwide and in Pakistan has been become a point of major discussion. The pest has gained substantial and intense levels of resistance to all major groups of pesticides such as carbamates, organochlorines, pyrethroids and organophosphates (Ahmad *et al.*, 1999; Regupathy *et al*., 2003; Ahmad, 2007). Application of most insecticides belongs to these groups against Lepidoptera pests particularly *Helicoverpa* spp. is still considered as the major issue in our region and regular toxicity effect of these and new arrived pesticides need to proper test in field and laboratory condition. Efforts were therefore made to find out the effects of different insecticides against gram pod borer.

In Pakistan, most pyrethoids such as cypermethrin are generally suggested that historically have proved effective in order to control many agricultural insect pests. However, few novel compounds such as Avermectin, Flubendiamide, Diflubenzuron recently provided an excellent result in controlling the pest population but still did not comparatively studied against *H. armigera* on chickpea (Abbas *et al.*, 2015). As most Asian farmers are economically poor and they could not afford high expensive and repetitive use of pesticides. The situation requires exploring such chemicals that can be cheaper and effective in controlling *H. armigera* population on chickpea. Therefore, the present study is a latest addition in already work done around the world regarding the performance of different pesticides against *H. armigera*. It is expected that the produced results will be

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helpful in selecting the better pesticide to control the pest population below economic threshold level. Therefore, the research was conducted to evaluate the compatibility of novel chemical compounds against *H. armigera* in field and laboratory conditions with the following objectives:

2. MATERIALS AND METHODS

Place of work

The experiment was conducted in field condition at Pulse Section, Agriculture Research Institute (ARI), Tando jam during the Rabi season, 2017-2018.

Experimental design and layout

The experiment was conducted in Randomized Complete Block Design (RCBD) with five treatments with 15 replications. The overall experimental size of area was 36x50 sq feet that further divided into 15 blocks (10 x 12 sq. feet) as mentioned as **(Fig. 1).** In each block, all pesticides were sprayed to know their effect on pod borer.

Four different pesticides were applied on *H. armigera* in field condition. The selected pesticides were Proclaim 2.1EC, Steward 16.5EC, Belt 39.4SC and Summit 2.7SC. The pesticides were applied according to their recommended dose and before their application, larval population were observed to know the economic threshold level (1 larva per square foot or per plant).

Calibration of pesticides for field application

Recommended dose was followed by printing on label of each pesticide bottle/product and further was calculated according to requirement per area. Eight knapsack sprayers (tanks) are generally recommended for 1 acre (43560 sq. feet) and each tank of sprayer containing 16 lit of water that was make 128 lit of water required to spray for an acre of agriculture land. Therefore, the sprayed dose of each pesticide was used/ calculated as mentioned below and further explained in **(Table 1).** Area of application for each treatment= row size x number of replications.

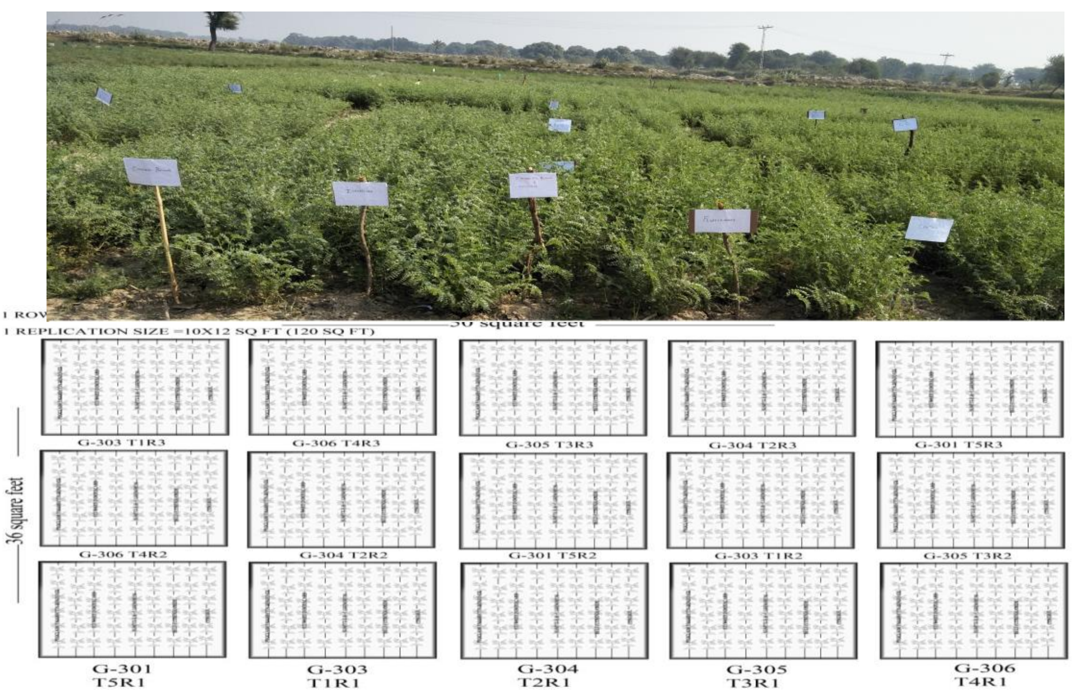

Fig.1. Field layout of chick pea for experimentation

Table 1. Selected pesticides used in experiment describing calculated dose from recommended dose

Trade Name	Common Name	Pesticide Group	Recommended Dose ml/acre	Requireddose ml/treatment
Emamectin Benzoate 2.1%EC	Proclaim	Avermectin	200 ml	0.68 ml
Indoxacarb 16.5%EC	Steward	Oxadiazine	90 ml	0.30 ml
Flubendiamide 39.4%SC	Belt	Ryanoid	20 ml	0.068 ml
Emamectin Benzoate + lufenoron 2.7%EC	Summit	Benzoylurea	400ml	1.37 ml

The spray was performed with hand compression sprayer as sprayed area was not selected bigger and the pesticide was required in small quantity. A glass beaker of 500 ml and 1 ml insulin syringe was used to measure the required quantity of pesticide and water. Keeping in view, all pesticide precaution measures, hand gloves, dust mask, wind flow, proper shoe and timing (early morning) was apply. The sprayed lines/plots were tagged properly (Figure 2) to make easiness for data collection

Experimental outlines

Before each spray, pre-treatment data in regard to observe the gram pod borer (larvae) per three plants in row randomly was examined. Later, the data were recorded after 24, 48, and 72hrs and one week, respectively after spray. Overall, three spray was carried with interval of 15 days.

Laboratory bioassay

To determine the lethal time LT_{50} and LT_{90} of *H. armigera* at different doses of pesticides under laboratory condition, a bioassay was carried out in a laboratory. Three concentrations of each pesticide was prepared (50 ppm, 100 ppm and 150 ppm) and applied to 3rd instar larvae of gram pod borer to observe their mortality. The larval instars were selected on their morphological character such as size. In experiment, the prepared dose of pesticides was applied on filter paper and left for drying. Later, the filter paper was placed in an ice cube (plastic tray) on which single larvae of 3rd instar separately introduced with few fresh leaves **(Fig.2).** The mortality percentage of 3rd instar larvae was observed after 12, 24, 36, 48, 60 and 72 hrs, respectively.

Fig.2. Bioassay to observe the effect of pesticides on larvae inside ice tray

Data analysis

The data were statistically analyzed using analysis of variance (ANOVA) and mean differences also compared by LSD test at $p<0.05$ in order to observe the effect of used pesticides in field condition. However, for bioassay, LT_{50} and LT_{90} tests were used by using Polo Plus (version: 0.03) software.

3. RESULTS

Pest population of *H. armigera* after first spray

After application of first spray, the population of *H. armigera* was observed statistically different ($P<0.05$) at 24 hrs, 48 hrs, 72 hrs and one week as mentioned in **(Table-2).** At 24hrs, the lowest population (0.44±0.11) was observed at Proclaim and the higher population (1.06±0.07) at Summit, respectively. At 48 hrs, the lowest population (0.46±0.10) was observed at Proclaim and the higher population (1.17±0.08) at Summit, respectively. At 72 hrs, the lowest population (0.48±0.04) was observed at Proclaim and the higher population (1.24±0.09) at Summit, respectively. At 1 week, the lowest population (0.51±0.09) was observed at Proclaim and the higher population (1.28±0.08) at Summit, respectively. However, overall the highest population of gram pod borer was observed in control treatment at all times. The best results in order to observe the effect of pesticide on population of gram pod borer was observed at Proclaim in all times.

Pest population of *H. armigera* after second spray

After application of second spray, the population of *H. armigera* was observed statistically different ($P<0.05$) at 24 hrs, 48 hrs, 72 hrs and 1 week as mentioned in **(Table-3).** At 24 hrs, the lowest population (0.28±0.03) was observed at Proclaim and the higher population (0.95±0.13) at Summit, respectively. At 48 hrs, the lowest population (0.31±0.06) was observed at Proclaim and the higher population (1.02±0.05) at Summit, respectively. At 72 hrs, the lowest population (0.35±0.6) was observed at Proclaim and the higher population (1.09±0.09) at Summit, respectively and at 1 week, the lowest population (0.37±0.03) was observed at Proclaim and the higher population (1.15±0.09) at Summit, respectively. However, overall highest population of gram pod borer was observed in control treatment. The best result in second spray was also observed at Proclaim in all times.

Pest population of *H. armigera* after third spray

After application of third spray, the population of *H. armigera* was observed statistically different ($P<0.05$) at 24 hrs, 48 hrs, 72 hrs and 1 week as mentioned in **(Table-4)**. At 24 hrs, the lowest population (0.17±0.04) was observed at Proclaim and the higher population (0.95±0.11) at Summit, respectively. At

48 hrs, the lowest population (0.19±0.05) was observed at Proclaim and the higher population (1.06±0.06) at Summit, respectively. At 72 hrs, the lowest population (0.24±0.03) was observed at Proclaim and the higher population (1.22±0.11) at Summit, respectively and at 1 week, the lowest population (0.26±0.04) was observed at Proclaim and the higher population (1.31±0.10) at Summit, respectively. However, overall highest population of gram pod borer was observed in control treatment at all times. The best result in third spray was also observed at Proclaim in all times.

Overall reduction percentage of different pesticides

After application of all spray, the overall reduction percentage of different insecticides was observed at 24 hrs, 48 hrs, 72 hrs and 1 week as mentioned in **(Table-5).** At 24 hrs, maximum reduction percentage of 63.92% was observed at Proclaim and minimum reduction percentage of 31.72% was observed at Summit, respectively. At 48 hrs, maximum reduction percentage of 62.03% was observed at Proclaim and minimum reduction percentage of 25.84% was observed at Summit, respectively. At 72 hrs, maximum reduction percentage of 60.00% was observed at Proclaim and minimum reduction percentage of 24.82% was observed at Summit, respectively. At 1-week, maximum reduction percentage of 60.90% was observed at Proclaim and minimum reduction percentage of 23.95% was observed at Belt, respectively. The best results in order to observe the population reduction of gram pod borer was observed at Proclaim followed by Steward at all times. However, Summit did not show significant effect on reducing pest population from field.

Table 2. Mean population (*Mean±S.E) of *H. armigera* on chickpea after first spray of different pesticides

Treatments	Pre-treatment	24 hrs	48 hrs	72 hrs	Week
Proclaim	1.44 ± 0.20ab	0.44 ± 0.11d	0.46 ± 0.10d	0.48 ± 0.04d	0.51 ± 0.09d
Steward	1.53 ± 0.20a	0.60 ± 0.09cd	0.64 ± 0.03cd	0.66 ± 0.08cd	0.75 ± 0.06c
Summit	1.64 ± 0.13a	1.06 ± 0.07b	1.17 ± 0.08b	1.24 ± 0.09b	1.28 ± 0.08b
Belt	1.04 ± 0.14b	0.75 ± 0.05c	0.77 ± 0.07c	0.80 ± 0.06c	0.84 ± 0.77c
Control	1.62 ± 0.14a	1.64 ± 0.12a	1.68 ± 0.11a	2.13 ± 0.10a	2.88± 0.09a

Table 3. Mean population (*Mean±S.E) of *H. armigera* on chickpea after second spray of different pesticides

Treatments	Pre-treatment	24hrs	48hrs	72hrs	Week
Proclaim	0.57 ± 0.06d	0.28 ± 0.03c	0.31 ± 0.06c	0.35 ± 0.6c	0.37 ± 0.03d
Steward	0.82 ± 0.07cd	0.51 ± 0.04c	0.53 ± 0.10c	0.60 ± 0.07c	0.66 ± 0.07cd
Summit	1.42 ± 0.08b	0.95 ± 0.13b	1.02 ± 0.05b	1.09 ± 0.09b	1.15 ± 0.09b
Belt	0.88 ± 0.08c	0.53 ± 0.05c	0.60 ± 0.09c	0.66 ± 0.08c	0.75 ± 0.05c
Control	3.33 ± 0.14a	3.37 ± 0.18a	3.42 ± 0.24a	3.44 ± 0.21a	3.60 ± 0.26a

Table 4. Mean population (*Mean±S.E) of *H. armigera* on chickpea after third spray of different pesticides

Treatment	Pre-treatment	24hrs	48hrs	72hrs	Week
Proclaim	0.44 ± 0.04d	0.17 ± 0.04d	0.19 ± 0.05d	0.24 ± 0.03d	0.26 ± 0.04d
Steward	0.75 ± 0.06cd	O.48 ± 0.04cd	0.51 ± 0.07c	0.57 ± 0.06cd	0.62 ± 0.09c
Summit	1.24 ± 0.06b	0.95 ± 0.11b	1.06 ± 0.06b	1.22 ± 0.11b	1.31 ± 0.10b
Belt	0.86 ± 0.08bc	0.57 ± 0.10c	0.67 ± 0.04c	0.82 ± 0.07bc	1.00 ± 0.09b
Control	3.66 ± 0.30a	3.71 ± 0.18a	3.75 ± 0.14a	3.88 ± 0.26a	3.99 ± 0.20a

*Means followed by different letters within the same column (Table 2-4) are significantly different (P<0.05)

Table 5. Overall reduction percentage of *H. armigera* on chickpea after three sprayers Using Henderson Tilton's formula

Treatment	24hrs	48hrs	72hrs	Week
Proclaim	63.92%	62.03%	60.00%	58.90%
Steward	49.25%	47.11%	46.04%	45.71%
Summit	31.72%	25.84%	24.82%	19.12%
Belt	34.25%	28.86%	25.54%	23.95%

Table 6. LT_{50} values (hours) of different concentrations against *H. armigera*

Treatments	Concentrations (ppm)	LT_{50}	95% confidence limits		Slope ± SE	X^2
			Lower	Upper		
Proclaim	50	17.72	10.51	23.49	2.77 ± 0.61	11.95
	100	13.70	5.65	19.60	2.77 ± 0.67	17.05
	150	10.30	0.73	18.34	1.59 ± 0.56	12.31
Steward	50	18.18	13.26	22.44	4.15 ± 0.81	3.81
	100	16.91	12.26	20.91	4.31 ± 0.88	15.63
	150	15.66	2.68	24.85	1.55 ± 0.54	10.44
Summit	50	27.82	11.41	42.59	2.5 ± 0.57	33.27
	100	26.51	15.93	35.96	2.09 ± 0.55	13.96
	150	21.73	15.63	27.10	3.39 ± 0.66	9.67
Belt	50	28.80	20.72	36.91	2.75 ± 0.61	6.06
	100	13.53	2.48	21.56	1.73 ± 0.57	11.92
	150	12.83	2.75	20.24	1.86 ± 0.59	6.47

Table 7. LT_{90} values (hours) of different concentrations against *H. armigera*

Treatments	Concentrations (ppm)	LT_{90}	95% confidence limits		Slope ± SE	X^2
			Lower	Upper		
Proclaim	50	51.25	38.45	88.54	2.77 ± 0.61	3.81
	100	39.74	28.75	76.38	2.77 ± 0.67	17.05
	150	65.26	40.67	403.01	1.59 ± 0.56	12.31
Steward	50	36.99	29.68	53.19	4.15 ± 0.81	11.95
	100	33.54	26.86	48.82	4.31 ± 0.88	15.63
	150	104.03	58.89	1309.24	1.55 ± 0.54	10.44
Summit	50	90.07	54.35	918.96	2.51 ± 0.57	33.27
	100	108.64	68.45	395.28	2.09 ± 0.55	13.96
	150	51.81	40.47	80.17	3.39 ± 0.66	9.67
Belt	50	84.20	59.49	184.44	2.75 ± 0.61	6.06
	100	70.49	5.12	23.09	1.95 ± 0.58	11.38
	150	62.71	2.75	20.24	1.86 ± 0.59	6.47

Bioassay in order to observe the lethal time (LT_{50} and LT_{90}) of *H. armigera* under laboratory condition

A laboratory bioassay experiment was carried out to evaluate the lethal time (LT_{50} and LT_{90}) using four different insecticides against pod borer *H. armigera*. These insecticides were applied at the dose of 50ppm, 100ppm and 150ppm were applied to confirm their lethal time against the target pest.

LT_{50} values (hours) of concentration of 50ppm, 100ppm and 150ppm against *H. armigera*

The LT_{50} was observed as mentioned in Table 6. At 50ppm, the highest LT_{50} was 28.80 hrs (slope 2.75 and Chi sq. 6.06), at Belt and the lowest 17.72 hrs (slope 2.77 and Chi Sq. 11.95) at Proclaim. At 100ppm the highest LT_{50} was 26.51 hrs (slope 2.09 and Chi sq. 13.96) at Summit and the lowest 13.70 hrs (slope 2.77 and Chi Sq. 17.05) at Proclaim. At 150ppm the highest LT_{50} was 21.73 hrs (slope 3.39 and Chi sq. 9.67) at Summit and the lowest 10.30 hrs (slope 1.59 and Chi Sq. 12.31) at Proclaim, respectively observed. However, the overall results showed a significant difference in all treatments at all selected intervals but Proclaim killed 50% population of *H. armigera* within lowest time.

LT_{90} values (hours) of concentration of 50ppm, 100ppm and 150ppm against *H. armigera*

The LT_{90} was observed at different doses and times as mentioned in Table 7. At 50ppm, the highest LT_{90} of 90.07 hrs (slope 2.51 and Chi sq. 33.27) was observed at Summit and the lowest 36.99 hrs (slope 4.15 and Chi Sq. 11.95) was observed at Steward respectively. At 100ppm, the highest LT_{90} of 108.64 hrs (slope 2.09 and Chi sq. 13.96) was observed at Summit and the lowest 33.54 hrs slope (4.31 and Chi Sq. 15.63) was observed at Steward respectively. At 150ppm, the highest LT_{90} of 104.03 hrs (slope 1.55 and Chi sq. 10.44) was observed at Steward and the lowest 51.81 hrs and (slope 3.39 and Chi Sq. 9.67) was observed at Summit respectively. However, overall a significant difference was observed in all treatments at all selected intervals.

4. DISCUSSION

The studies were undertaken to know the toxicity of novel chemical compounds against *H. armigera* in field and laboratory conditions. The results obtained are discussed in the light of available literature. The gram pod borer (*H. armigera*) is a serious insect pest and supposes to cause considerable loss to chickpea production (Deshmukh *et al.,* 2010). The pest causes heavy losses in field crops, horticultural crop as well as vegetable crops in the country and throughout the world (Fitt, 2008). Insecticide usage is general method of eradication for insect pests particularly for *H. armigera*. A number of insecticides registered for control of insect pests in Pakistan which includes huge list of synthetic insecticide (i.e. profenofos, endosulfan, methomyl, thiodicarb, azinophos, flucythrinate, tralomethrin, esfenvalerate, beta-cyfluthrin, zeta-cypermethrin, lambda-cyhalothrin and indoxcarb) bu t the overuse of these insecticides causes secondary outbreaks and development of pests (Ahmad *et al.,* 2007). The resistance and outbreak population *H. armigera* resistance against many insecticides has been well documented in the world by a number of researchers including in Pakistan (Ahmad *et al.,* 2007).

In present study, worldwide well known pesticides were used against this noxious pest. The best findings in field and laboratory condition were observed at Proclaim with maximum reduction in pest population (63.92%) at different time intervals. These results are partially in agreement with those of who also observed productive results in order to reduce gram pod borer population by using Proclaim. However, Steward is a broad-spectrum insecticide in a new class of chemical compound with a novel type to kill the lepidopteran pests such as *H. armigera* and *S. exigua*. Steward actually not a systemic pesticide and has translaminr movement into the mesophyll. Steward affects insect through direct revelation to spray droplets and through ingestion of treated foliage/fruit. Once absorbed, it kills by binding to a site on the sodium channel and blocking the flow of sodium ions. The result is impaired nerve function, feeding cessation, paralysis and death. Beneficial insects such as damsel bug, predacious mites, big eyed bug, minute pirate bug, assassin bug, green lacewing larvae, spiders, and parasitic wasps are not significantly affected by the residues of Steward. This is primarily because of very limited ingestion due to the feeding habits of these insects and lack of uptake via tarsal exposure (McKinley *et al.,* 2002).

After productive efficacy of Proclaim, a significant effect in term of reducing pest population of *H. armigera* was also observed at Steward (49.25%). The same results were observed by Rani *et al.* (2005) with productive efficacy of Steward. They also conduct similar experiment for test Indoxacarb and Spinosad and get similar results against *H. armigera* and they found indoxacarb more toxic to pod borer than spinosad. Belt belongs to diamide group of insecticides having a mode of action by activating the ryanodine receptors (RyRs), depletion of internal Ca resulted due to uncontrolled release, general lethargy, muscle paralysis, regurgitation accompanied with rapid feeding cessation ultimately causing death within 72 hours (Carlson *et al*., 2001; Dow Agro Sciences, 2003; Teixeira and Andaloro, 2013). Meanwhile, the results of present study also endorsed the late effect of Belt in field and laboratory condition. All used pesticides displayed their quick effect particularly at 24 hrs in term of reducing pest population and thus effect reduced with time but effect of Proclaim and Steward remain productive until week and even though pest did not read at economic threshold level after fortnight of first spray.

Apart from two well reputed insecticides (Proclaim and Steward) belongs to multinational companies, another novel pesticides (Belt) was also used in field and laboratory experiments. Even tough, the results of Belt against *H. armigera* was fewer than first two pesticides but still it gave significant results in term of pest reduction from field condition (34.25%) as compared to Summit. The new chemistry insecticide, Belt (flubendiamide) was also found effective on the basis of lower pod infestation with higher level of yield due to significant percent mortality of pest. The results are partially in agreement with those of (Ameta and Bunker 2007; Tatagar *et al*. 2009; Meena *et al*. 2013; Sreekanth *et al*. 2014; Karar *et al*. 2017). Meanwhile, Summit showed less significant reduction in pest population (31.72%) these results are partially in agreement with those of (Patil *et al*. 2007; Iqbal *et al*. 2014; Hakeem *et al*. 2017) who recorded less effect of emamectin benzoate with mixed sprayed with lufenoron.

5. CONCLUSION

The best results in order to observe the population reduction of gram pod borer was observed at Proclaim followed by Steward at all times in field and lab conditions. However, Summit did not show significant effect on reducing pest population from field. Although, Belt showed its toxicity in field and laboratory condition but on higher dose and with late effect. Furthermore, it was also noticed that Proclaim does not need many sprays as after application of first spray, the ETL level was not as high as required for other pesticides.

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