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Laboratory evaluation of toxicity of selected insecticides against egg and larval stages of cotton pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae)

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Abstract

Background The cryptic nature of pink bollworm *Pectinophora gossypiella* (Saunders) larvae enables its reduced vulnerability to insecticidal control. Further, the development of resistance against *Bacillus thuringiensis* (Bt) toxins posed a serious threat to transgenic cotton cultivation. This necessitated determining the critical timing of spray applications on the control effectiveness. This study assessed the influence of egg age (freshly laid *vs*. three-day-old) and the location of larvae (directly exposed to the insecticide residues on the boll rind *vs*. burrowed inside the bolls) on insecticide control efficacy.

Results The results revealed a significant decrease in the ovicidal activity of tested insecticides with an increase in the age of eggs from one day old to three days old (paired *t*-test, $P < 0.05$). The larvae directly exposed to the insecticide residues on the boll rind were more susceptible (> 80% mortality) than the larvae exposed after they had burrowed inside the bolls (< 49% mortality). The inhibitory effects of tested insecticides on developmental biology were more pronounced in the experiment on pre-larval release insecticide treatment compared with insecticide treatment given post-larval release and entry inside the bolls.

Conclusion Egg age influences the insecticide susceptibility, as does the larval location, directly exposed vs burrowed inside the bolls. Older eggs and the larvae that had burrowed inside the green bolls of cotton were relatively less susceptible to the insecticide treatments. The toxic effects of insecticides on egg and larval stages were primarily ephemeral. These findings are significant for devising a comprehensive strategy for pink bollworm management on a sustainable basis.

Keywords Bioefficacy, Cotton, Insecticides, Pink bollworm, *Pectinophora gossypiella*, Timings of spray

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Background

Cotton (*Gossypium* spp.), plays an important role in the Indian and international economies, both in terms of employment generation and foreign exchange (Directorate of Cotton Development 2017). Cotton is grown mainly for its fibre in more than seventy countries/

regions of the world of which major ones are India, China, USA, Brazil, and Pakistan, accounting for approximately three-quarters of the world's cotton production (Statista 2020). Globally, cotton is grown in an area of 38.64 million ha with a production of 82.59 million tones (Food and Agricultural Organization STAT 2019). In India, cotton is cultivated in an area of 13.28 million ha with a production of 35.25 million bales (170 kg per bale) and lint productivity of 451.0 kg·ha^{-1} as against the world average of 712.0 kg·ha^{-1} (The Cotton Corporation of India 2022; Directorate of Economics and Statistics 2021).

The pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), is a highly destructive insect pest of cotton across the global cotton-producing areas. The probable origin of pink bollworm is in the Indo-Pakistan region (Saunders 1843). It is now present in tropical America, Africa, Asia, and Australia (Commonwealth Agricultural Bureau International (CABI) 2022). The life cycle of pink bollworm passes through four distinct developmental stages, viz., egg, larva, pupa, and adult, and requires accumulation of ~ 500 growing degree days (GDD) for completion of one generation (Beasley et al. 1996; Peddu et al. 2020; Fand et al. 2021). The length of the life cycle of pink bollworm varies with prevailing environmental conditions, especially the temperature during the cotton growing season, being shortest (35~37 d) during relatively warmer months of July to October, and longest (59~73 d) during cooler winter months of November to next January (Peddu et al. 2020; Fand 2021; Fand et al. 2021). Generally, pink bollworm is a late-season pest of cotton, its infestation coincides with the onset of squaring, flowering, and boll development (Fand 2021; Fand et al. 2021); accounting for huge yield losses to the tune of 30% (Fand et al. 2019). The larvae of pink bollworm feed on developing flower buds and seeds of green bolls of the cotton plant, which causes rosette flowers, premature opening of infested bolls, reduction in fibre length and poor quality of lint due to staining (Fand et al. 2019 2020; Fand 2021).

Pink bollworm has re-emerged as a major pest in Indian cotton production, mainly due to the development of resistance against transgenic cotton expressing *cry*1*Ac* and *cry*2*Ab*2 genes from the entomopathogenic bacterium *Bacillus thuringiensis* Kurstaki (Dhurua et al. 2011; Naik et al. 2018). Re-emergence of pink bollworm has posed a serious threat to the continued success of genetically-modified cotton in combating serious bollworm pests. The widespread infestation of pink bollworm causing yield losses between 10%~30% was reported in central and southern cotton growing belts of India during 2017/2018 (Fand et al., 2019). Incipient infestations of pink bollworm on Bt cotton in the North Zone have been reported since 2018/2019 (Kumar et al. 2020). However, the strong outbreak of this pest caused huge losses to the Bt cotton crop during the 2021/2022 growing season (Prasad et al. 2022). These circumstances have left farmers with no option other than to resort to chemical insecticides as during the pre-Bt era, for managing the pink bollworm infestations in their cotton crop. Before the introduction of transgenic cotton, synthetic pyrethroids played a major role in the management of the bollworm complex of cotton (Kranthi et al. 2002). Insecticides are generally considered as the first line of defence against insect pests by farmers. Currently, the pink bollworm management in cotton in India is highly reliant on the use of chemical insecticides. About 21% of total pesticide use in India is accounted for managing various pest problems in cotton (Kranthi 2012), and a major portion of it is shared by pink bollworm alone. However, the use of insecticides for pink bollworm management is often futile because of its cryptic habits that the entire development of larvae is completed inside the infested bolls of cotton (Fand et al. 2020; CABI 2022). Even larvae of pink bollworm remain concealed inside infested/rosette flowers and are thus protected from direct exposure to insecticides. These results in repeated insecticide applications by the farmers to achieve the desired level of pest control which ultimately escalates the other side effects like the development of insect resistance, environmental contamination, and increased cost of protection (Bajya et al. 2010).

The timing of insecticide application, synchronized with pest population status, monitoring methods, strict surveillance, etc., is highly important. The blanket application of periodical, calendar date-based insecticide sprays seems to be unwise in suppressing the pink bollworm because of increased target site inaccessibility of the spray chemicals once the larvae penetrate the bolls. Therefore, for better control efficacy, the insecticide sprays should be applied well before the larvae enter the green bolls (Fand 2021; Fand et al. 2021). In this context, some insecticides and their combination products were tested at their recommended field doses (Directorate of Plant Protection, Quarantine and Storage 2022) against egg and larval stages of pink bollworms under laboratory conditions. The effect of egg age and larval location (directly exposed to insecticide residues on boll rind *vs* burrowed inside the green bolls) on the effectiveness of insecticidal control of pink bollworms was investigated.

Results

Ovicidal effect of insecticides

The results revealed that all the tested insecticides when used at their recommended doses for field application had a profound influence on the hatching of freshly laid

(< 8 h old) eggs of pink bollworms (< 23.00% egg hatching) compared with the untreated control (82.67% egg hatching) (Table 1). The treatments of neem seed kernel extract (NSKE) 0.15% EC (emulsifiable concentrate) and ethion 50% EC registered the strongest ovicidal action with minimal egg hatching of 16.00% in both cases. Except for emamectin benzoate 5% SG (water soluble granule), all others were at par, with egg hatching ranging from 17.33% to 22.67%. The treatment of emamectin benzoate 5% SG with relatively more egg hatching (52.00%) was found as the least effective ovicide. The tested insecticides with a descending order of their ovicidal action against freshly laid eggs, as revealed from the reduction in egg hatching, were: NSKE 0.15% EC (80.61%) = ethion 50% EC (80.61%) > lambda-cyhalothrin 5% EC (78.94%) > chlorpyrifos 50% + cypermethrin 5% EC (75.91%) = ethion 40% + cypermethrin 5% EC (75.91%) > profenophos 50% EC (75.76%) > cypermethrin 25% EC (72.42%) = profenophos 40% + cypermethrin 4% EC (72.42%) > emamectin benzoate 5% SG (36.82%).

All the tested insecticides significantly influenced the hatching of three-day-old (~ 72 h) eggs of pink bollworms. However, the ovicidal action of the tested insecticides is reduced as revealed by increased egg hatching (< 38.00%) compared with the egg hatching recorded in freshly laid eggs (< 23.00%) (Table 1). The strong ovicidal activity against three-day-old eggs of pink bollworms was observed in the treatments of ethion 40% + cypermethrin 5% EC (14.67% egg hatching), lambda-cyhalothrin 5% EC (16.00%), and cypermethrin 25% EC (16.00%). The rest of the tested insecticides (barring NSKE 0.15% EC) recorded egg hatching in the range of 22.67%~29.33% and were found as effective ovicides against three-day-old eggs of pink bollworms. As revealed from the increased egg hatching (37.33%), the treatment of NSKE 0.15% EC had reduced ovicidal action against three-day-old eggs of

Table 1 Ovicidal effect of selected insecticides against cotton pink bollworm in laboratory

Treatment No	Treatment details	Sample size*	Egg hatching ± SE /%#		Reduction in egg hatching compared to control ± SE /%#	
			Freshly laid eggs (< 8 h old)	Three-day-old eggs (~ 72 h old)	Freshly laid eggs (< 8 h old)	Three-day-old eggs (~ 72 h old)
T1	Profenophos 50% EC	75	20.00 ± 2.31^{ab} (26.49 ± 1.66)	24.00 ± 2.31^{bc} (29.28 ± 1.55)	75.76 ± 2.98^{ab} (60.58 ± 1.98)	71.10 ± 1.60^{bc} (57.50 ± 1.01)
T2	Lambda-cyhalothrin 5% EC	75	17.33 ± 1.33^{a} (24.57 ± 1.00)	16.00 ± 2.31^{a} (23.47 ± 1.82)	78.94 ± 2.04^{a} (62.73 ± 1.42)	80.33 ± 3.67^{a} (63.86 ± 2.66)
T3	Emamectin benzoate 5% SG	75	52.00 ± 2.31^{c} (46.15 ± 1.33)	29.33 ± 1.33^{c} (32.78 ± 0.83)	36.82 ± 4.55^{c} (37.29 ± 2.70)	64.49 ± 1.10^{c} (53.43 ± 0.66)
T4	Ethion 50% EC	75	16.00 ± 0.00^{a} (23.58 ± 0.00)	22.67 ± 1.33^{b} (28.41 ± 0.92)	80.61 ± 0.61^{a} (63.88 ± 0.44)	72.61 ± 0.65^{b} (58.45 ± 0.42)
T5	Cypermethrin 25% EC	75	22.67 ± 1.33^{b} (28.41 ± 0.92)	16.00 ± 4.00^{a} (23.29 ± 3.02)	72.42 ± 2.42^{b} (58.37 ± 1.58)	80.17 ± 5.88^{a} (63.98 ± 4.09)
T6	Neem seed kernel extract (NSKE) 0.15% EC	75	16.00 ± 2.31^{a} (23.47 ± 1.82)	37.33 ± 3.53^{d} (37.62 ± 2.09)	80.61 ± 2.95^{a} (63.99 ± 2.12)	54.63 ± 4.73^{d} (47.69 ± 2.74)
T7	Profenophos 40% + Cypermethrin 4% EC	75	22.67 ± 2.67^{b} (28.36 ± 1.79)	25.33 ± 3.53^{bc} (30.12 ± 2.31)	72.42 ± 3.77^{b} (58.42 ± 2.39)	69.20 ± 4.45^{bc} (56.41 ± 2.80)
T8	Chlorpyrifos 50% + Cypermethrin 5% EC	75	20.00 ± 2.31^{ab} (26.49 ± 1.66)	25.33 ± 1.33^{bc} (30.21 ± 0.87)	75.91 ± 2.15^{ab} (60.65 ± 1.46)	69.10 ± 3.00^{bc} (56.28 ± 1.84)
T9	Ethion 40% + Cypermethrin 5% EC	75	20.00 ± 2.31^{ab} (26.49 ± 1.66)	14.67 ± 1.33^{a} (22.47 ± 1.10)	75.91 ± 2.15^{ab} (60.65 ± 1.46)	82.09 ± 2.21^{a} (65.05 ± 1.69)
T10	Control (water spray)	75	82.67 ± 2.67^{d} (66.53 ± 2.10)	82.67 ± 3.53^{e} (65.61 ± 2.65)	0.01 ± 0.00^{d} (0.57 ± 0.00)	0.01 ± 0.00^{e} (0.57 ± 0.00)
F test			sig**	sig**	sig**	sig**
SE (diff.) ±			2.14	2.65	2.46	3.07
CD at 5%			4.46	5.53	5.14	6.40
CV /%			8.19	10.03	5.72	7.18

SE is standard error, *CD* is critical difference, and *CV* is coefficient of variation

In parentheses are arc sin transformed values

** *F* test is highly significant at 1% level of significance

* Represents total sample size per treatment (25 eggs per replication)

a, b, c, d, e The same letter as superscript to values within a column indicate that the treatments are not significantly different from each other

pink bollworm in contrast to freshly laid eggs (16.00%). A reverse trend has been observed in the case of emamectin benzoate 5% SG, wherein the egg hatching has dropped from 52.00% in freshly laid eggs to 29.33% in three-day-old eggs, indicating the increased ovicidal action with the age of eggs. Based on the reduction in egg hatching, the tested insecticides can be arranged in a descending order of their ovicidal action against three-day-old eggs as ethion 40% + cypermethrin 5% EC (82.09%) > lambda-cyhalothrin 5% EC (80.33%) > cypermethrin 25% EC (80.17%) > ethion 50% EC (72.61%) > profenophos 50% EC (71.10%) > profenophos 40% + cypermethrin 4% EC (69.20%) > chlorpyrifos 50% + cypermethrin 5% EC (69.10%) > emamectin benzoate 5% SG (64.49%) > NSKE 0.15% EC (54.63%).

Application of *t*-test to the data on egg hatching recorded in various insecticide treatments separately to the freshly laid eggs and three-day-old eggs of pink bollworm revealed that the age of host eggs had significantly affected the ovicidal efficacy of all tested insecticides ($P < 0.03$ in all cases) except the lambda-cyhalothrin 5% EC ($P = 0.55$) and profenophos 40% + cypermethrin 4% EC ($P = 0.42$) (Table 2). The highest improvement in the egg hatching reduction effects against the ~ 72 h old eggs in comparison to the fresh eggs (~ 8 h old) was observed in the treatment of emamectin benzoate 5% SG followed by ethion 40% + eypermethrin 5% EC and cypermethrin 25% EC. The rest of the insecticides registered a significant decline in the egg hatching reduction effects against the ~ 72 h old eggs, with the highest decline in the treatment of NSKE0.15% EC.

Besides the numerical reduction in egg hatching, the tested insecticides also exhibited various morphological defects or aberrations in the treated eggs of pink bollworms. These effects included but were not limited to shrinking of egg chorion, yellowing and dark brown coloration of eggs, deformed eggs with dorsal longitudinal depression on the egg chorion, etc. Such morphological aberrations preventing the successful hatching of larvae from the insecticide-treated eggs support strongly the numerical observations recorded on the reduction in egg hatching in various insecticide treatments compared with the untreated control.

Larvicidal effect of insecticides

When the insecticide treatments to the green bolls were given before the larval release, more than 75.00% larval mortality was recorded after 24 h of exposure to all the tested insecticides. The treatment of NSKE 0.15% EC had recorded relatively lower mortality in neonate larvae of pink bollworm (76.62%) compared with the rest of the eight insecticides in which > 93.00% larval mortalities were recorded after 24 h of larval exposure. Compared with the untreated control (23.00%), all the tested insecticides were found as highly promising larvicides (Table 3). The fewer number of larvae that survived the insecticide exposure and entered the bolls by passing through a thin film of insecticide residues on the boll surface have failed to complete their development as either pupae or adult moths in all the tested insecticides resulting in the cent percent (100%) mortality, barring the treatment of NSKE 0.15% EC wherein the survivors (23.38%) could develop into the next stage. However, the other inhibitory effects on the developmental biology of pink bollworm observed in the treatment of NSKE 0.15% EC were: prolonged immature developmental duration (33.67 ± 0.63 d)

Table 2 Parameter estimates of paired *t*-test applied to the ovicidal effect of insecticides against cotton pink bollworm as influenced by the age of eggs (< 8 h old and ~ 72 h old)

Treatment No	Treatment details	Parameter estimates of paired *t*-test for egg hatching /%		
		df	*t* value	Significance (*P*)
T1	Profenophos 50% EC	10	40.90	< 0.000 01
T2	Lambda-cyhalothrin 5% EC	10	-0.64	0.55[#]
T3	Emamectin benzoate 5% SG	10	-13.48	0.000 04
T4	Ethion 50% EC	10	8.30	0.000 4
T5	Cypermethrin 25% EC	10	-3.02	0.03
T6	Neem seed kernel extract (NSKE) 0.15% EC	10	7.08	0.000 9
T7	Profenophos 40% + Cypermethrin 4% EC	10	0.88	0.42[#]
T8	Chlorpyrifos 50% + Cypermethrin 5% EC	10	3.16	0.025
T9	Ethion 40% + Cypermethrin 5% EC	10	-7.29	0.000 8
T10	Control (water spray)	10	0.04	0.97[#]

[#] *t*-test is non-significant

Table 3 Larvicidal effect of insecticides against neonate larvae of cotton pink bollworm

Treatment No	Treatment details	Sample size (*n*)*	Corrected larval mortality /%#		Reduction in insecticide efficacy due to larval entry inside the bolls /%
			Insecticide treatment to the green bolls prior to larval release	Insecticide treatment to the green bolls after release and entry of larvae inside the bolls	
T1	Profenophos 50% EC	75	93.74 ± 6.25^{a} (81.00 ± 8.33)	36.64 ± 2.90^{a} (37.22 ± 1.73)	60.91
T2	Lambda-cyhalothrin 5% EC	75	98.14 ± 1.85^{a} (85.00 ± 4.31)	24.39 ± 3.09^{bc} (29.50 ± 2.09)	75.15
T3	Emamectin benzoate 5% SG	75	99.98 ± 0.00^{a} (89.34 ± 0.04)	24.39 ± 3.09^{bc} (29.50 ± 2.09)	75.61
T4	Ethion 50% EC	75	99.98 ± 0.00^{a} (89.34 ± 0.04)	26.47 ± 1.47^{bc} (30.95 ± 0.95)	73.52
T5	Cypermethrin 25% EC	75	97.21 ± 2.77^{a} (83.99 ± 5.39)	28.43 ± 3.43^{b} (32.15 ± 2.15)	70.75
T6	Neem seed kernel extract (NSKE) 0.15% EC	75	76.62 ± 4.25^{b} (61.26 ± 2.87)	10.17 ± 1.97^{d} (18.41 ± 1.98)	86.72
T7	Profenophos 40% + Cypermethrin 4% EC	75	99.98 ± 0.00^{a} (89.34 ± 0.04)	36.76 ± 3.68^{a} (37.28 ± 2.18)	63.23
T8	Chlorpyrifos 50% + Cypermethrin 5% EC	75	99.98 ± 0.00^{a} (89.34 ± 0.04)	36.76 ± 0.74^{a} (37.32 ± 0.44)	63.23
T9	Ethion 40% + Cypermethrin 5% EC	75	99.98 ± 0.00^{a} (89.34 ± 0.04)	22.30 ± 3.55^{c} (28.05 ± 2.39)	77.69
T10	Control (water spray)	75	$0.01 \pm 0.00^{c\$}$ (0.57 ± 0.00)	$0.01 \pm 0.00^{e\&}$ (0.57 ± 0.00)	0.00
F test			sig**	sig**	
SE (diff.) ±			5.00	2.52	
CD at 5%			10.44	5.26	
CV /%			8.08	11.00	

* Represents total sample size, i.e., 75 larvae (25 larvae per replication)

In parentheses are arc sin transformed values (Gomez et al. 1984)

** *F* test is highly significant at 1% level of significance. The corresponding control mortalities used for calculating corrected mortalities in pre- and post-larval release treatments were: 23.33 ± 8.82% and 18.33 ± 1.67%

a, b, c, d, e The same letter as superscript to values within a column indicate that the treatments are not significantly different from each other

compared with the untreated control (23.58 ± 0.34 d) (Fig. 1), reduced longevities of adult moths (male 4.75 ± 0.52 d and female 6.00 ± 0.27 d) compared with the untreated control (male 11.39 ± 1.10 d and female 14.21 ± 1.57 d) (Fig. 2), and complete inhibition of oviposition as against 114.63 ± 17.60 eggs per female in the untreated control (Fig. 3).

Relatively lower mortality (< 37.00%) in neonate larvae of pink bollworm was observed when the insecticide treatments to the green bolls were given after the release and entry of larvae within the green bolls. The treatment of NSKE 0.15% EC recorded the lowest larval mortality (10.17%), whereas the larval mortalities in the rest of the eight chemical insecticides ranged between 22.30%~36.76%. The reduction in the efficacy of tested insecticides in killing the larvae of pink bollworm due to the larvae entering inside the boll ranged from the lowest value of 60.91% in the treatment of profenophos 50% EC to the highest value of 86.72% in the treatment of NSKE 0.15% EC (Table 3). No significant differences in the combined developmental durations of surviving larvae and pupae were observed in any of the tested treatments when the insecticides were applied after the larvae had burrowed inside the bolls (Fig. 1). However, most of the tested insecticides (except cypermethrin 25% EC) had significantly affected the longevity of male adults developed from larval survivals of insecticide treatments (reduction in adult survival time in the range of 14.00%~31.41%) (Fig. 2a). The longevity of female adults developed from surviving larvae in the treatments of profenophos 50% EC, cypermethrin 25% EC, and chlorpyriphos 50% + cypermethrin 5% EC was on par with that of the untreated control, whereas the rest of the insecticides could reduce the female adult longevity by 22.45%~35.42% (Fig. 2b). The results also indicated a significant effect of various insecticide

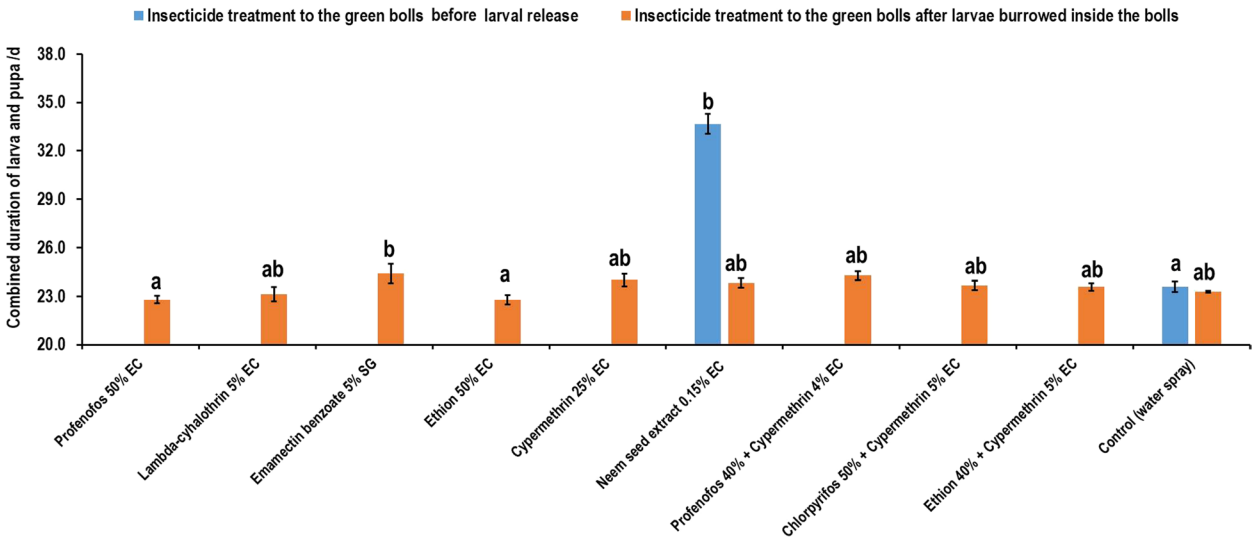

Fig. 1 Effects of various insecticide treatments on the developmental durations of surviving larval and pupal stages of cotton pink bollworm: The blue-colored bars indicate the results of insecticides applied before larval release and the orange-colored bars represent the results of insecticides applied after the larvae had burrowed inside the bolls. The different letters superscripts to the same colored bars indicate that the treatments are significantly different from each other. The means were separated by Tukey's Least Significant Difference (LSD) test at $P \leq 0.05$ level of significance

treatments on the fecundity of females developed from the larval survivors of insecticide applications, resulting in the reduction of female fecundity in the range of 14.27%~42.07% (Fig. 3).

Discussion

Pink bollworm being an internal feeder, the timings of insecticide applications are highly crucial in exercising its effective control. This is because the entire larval period of the pest is completed inside the bolls and once the larvae enter the bolls, the application of insecticide becomes ineffective or useless (Fand et al. 2020 2021). The calendar-based applications thus hold the least significance as they may not always exactly coincide with the egg laying by female moths or the hatching of larvae from the eggs under field conditions. Considering the combined mating and preoviposition period of 2~3 d and egg incubation period of 4~5 d for pink bollworm (Fand et al. 2020; Peddu et al. 2020), once the moth emergence is started, a very narrow window of 7~8 d remains available with the cotton growers to undertake the insecticide sprays for effective management of pink bollworm (Fand 2021; Fand et al. 2021).

Our results indicated that the ovicidal action of tested insecticides was decreased with the increase in the age of pink bollworm eggs in all the tested insecticides except emamectin benzoate 5% SG. This decreasing effect was more pronounced for the insecticides with greater ovicidal action (NSKE 0.15% EC) and moderately pronounced for the insecticides having both ovicidal and larvicidal actions (profenophos 50% EC, lambda-cyhalothrin 5% EC, ethion 50% EC, cypermethrin 25% EC, profenophos 40% + cypermethrin 4% EC, chlorpyrifos 50% + cypermethrin 5% EC, and ethion 40% + cypermethrin 5% EC). Our findings of newly laid eggs of pink bollworms being more sensitive than older ones to the insecticidal treatments are in strong agreement with earlier reports on the eggs of cotton bollworm, *Helicoverpa armigera* (Basavanneppa et al. 2014), and pink bollworm *Pectinophora gossypiella* (Sabry et al. 2018). We presume that the decrease in the ovicidal activity of insecticides with the increasing age of pink bollworm eggs may be because of the advancement of embryonic development akin to that reported by EL-Guindy et al. (1983).

According to the modes of action classes given by the Insecticide Resistance Action Committee (IRAC), NSKE 0.15% EC with azadirachtin as its principal active ingredient stands as an insect growth regulator with an unknown mode of action. Rest all insecticides used in the present study are nerve poisons at the molecular level (Sparks et al. 2015). The promising ovicidal effects observed in the present study under laboratory conditions may be attributed to the use of field doses of the insecticides for treating the eggs, which are relatively higher than those normally used under laboratory conditions. These findings indicate an ephemeral ovicidal activity similar to that observed in acetamiprid, thiodicarb, and lambda-cyhalothrin

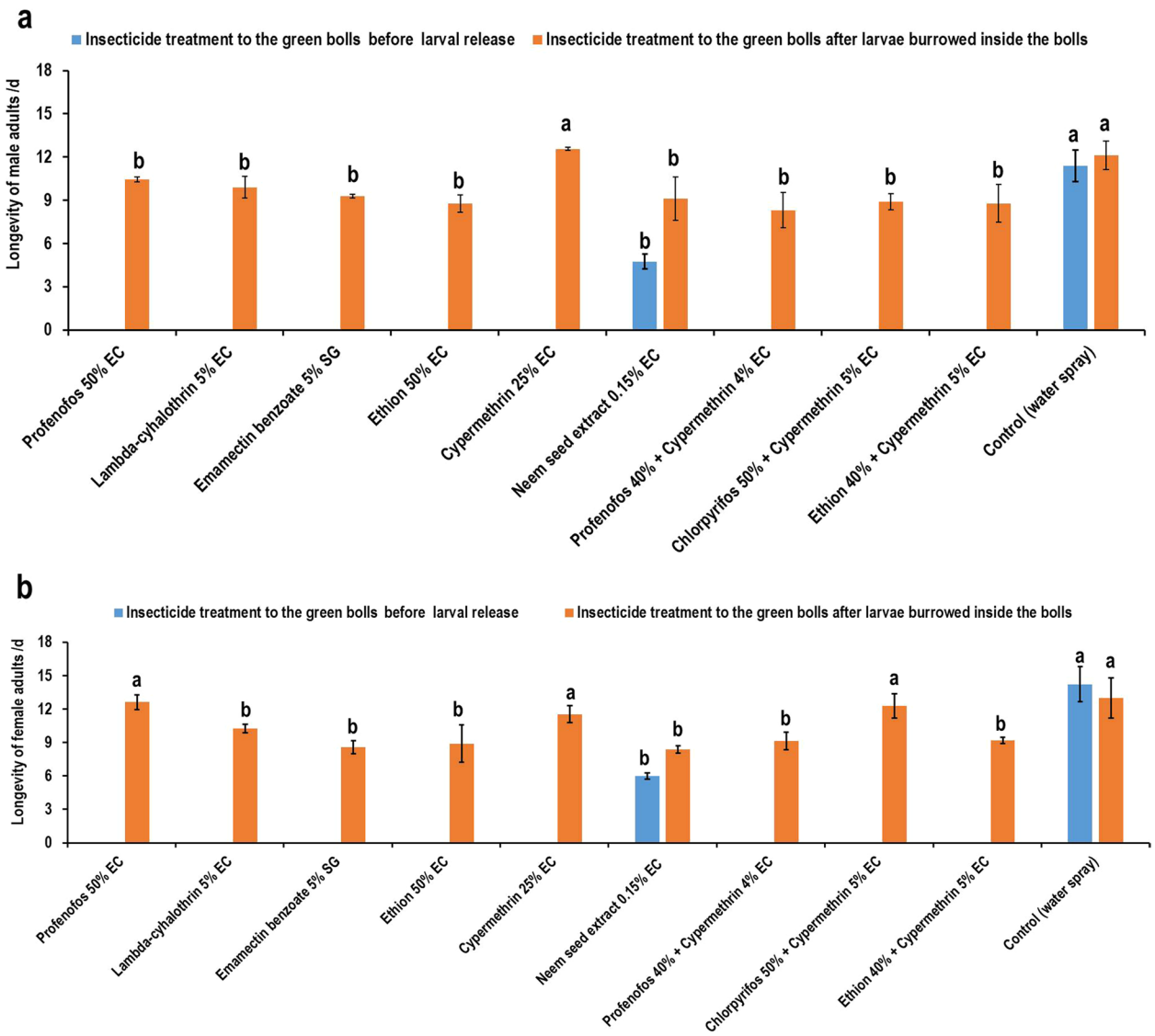

Fig. 2 The survival times of male (**a**) and female (**b**) adults developed from larval survivals of insecticide treatments: The blue-colored bars indicate the results of insecticides applied before larval release and the orange-colored bars represent the results of insecticides applied after the larvae had burrowed inside the bolls. The different letters superscripts to the same colored bars indicate that the treatments are significantly different from each other. The means were separated by Tukey's Least Significant Difference (LSD) test at $P \leq 0.05$ level of significance

against the eggs of cotton bollworm, *Helicoverpa armigera* (Ambrose et al. 2002). The marked decline in ovicidal activity with the advancement of egg age observed in the present study suggests that the use of insecticides that exhibit only ovicidal activity against pink bollworm seems to be impractical in cotton fields of India where the pest has re-emerged as a serious menace due to development of resistance to Bt toxins. Therefore, use of the insecticides that possess multiple effects like ovicidal, larvicidal, and oviposition deterrent actions coinciding with the moth emergence and oviposition activities can be the wise option for effective control of pink bollworms.

Nevertheless, we have noticed a reverse trend in the case of emamectin benzoate 5% SG, wherein the increased egg mortality was recorded in three-day-old eggs compared with freshly laid eggs of pink bollworms. Our results are in accordance with the findings of Moustafa (2016), who reported increased susceptibility of three-day-old eggs of pink bollworm over one-day-old eggs to emamectin benzoate 1.5% EC. The lower mortality observed in the freshly laid pink bollworm eggs treated with emamectin

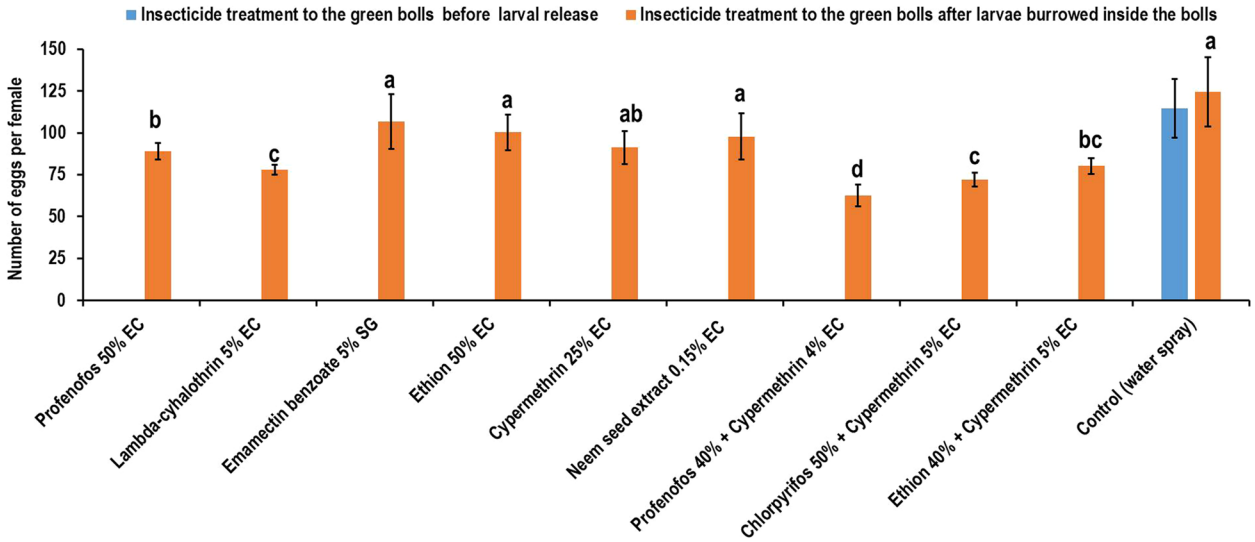

Fig. 3 Fecundity of female adults of cotton pink bollworm developed from larval survivals of insecticide treatments: The blue-colored bars indicate the results of insecticides applied before larval release and the orange-colored bars represent the results of insecticides applied after the larvae had burrowed inside the bolls. The different letters superscripts to the same colored bars indicate that the treatments are significantly different from each other. The means were separated by Tukey's Least Significant Difference (LSD) test at $P \leq 0.05$ level of significance

benzoate 5% SG in the present study could be explained by its marked larvicidal effect. Emamectin benzoate is a well-known stomach poison with substantial contact toxicity (Fanigliulo et al. 2008), and at the molecular level it acts as a nerve and muscle poison according to an IRAC mode of action classification (Sparks et al. 2015). This specifies that emamectin benzoate to be effective, it must be ingested by the target insect having well-developed muscular and nervous systems. The egg stage of insects lacks these systems which are developed primarily post-embryogenesis (Burrows 1996). This could be the reason for the selective toxicity of emamectin benzoate to three-day-old eggs of pink bollworms which are at the advanced stage of embryonic development over the freshly laid eggs. After hatching, the neonate larvae feed on the egg chorion and thus ingest the remains of the egg before moving to the target feeding site on the host plant (Guo et al. 2017). Feeding neonates on the chorions of insecticide-treated eggs might lead to their death.

The larvae directly exposed to the insecticide residues on the boll rind were more susceptible than the larvae that had already burrowed inside the bolls. When the treatments were given to the green bolls before larval release, all the tested insecticides provided significant control of the pink bollworm larvae with > 94.00% mortality, except the treatment of NSKE 0.15% EC (81.67%). The results indicated that the choice of insecticides for pink bollworms in the present experiment was appropriate as they have been reported as effective compounds in controlling cotton bollworms, e.g., chlorpyrifos for the control of pink bollworm and *Earias* spp. in cotton (Dhawan et al. 1989); cypermethrin against pink bollworm (Swamy et al. 2000), emamectin benzoate against the larvae of pink bollworm and *Earias insulana* (Boisd.) (Saleh et al. 2013); emamectin benzoate and lambda-cyhalothrin against the larvae of cotton bollworms (Patel 2013); profenophos, lambda-cyhalothrin, cypermethrin, and chlorpyriphos against pink bollworm in cotton (Zaki et al. 2015); profenophos against the larvae of pink bollworm (Moustafa et al. 2019). However, unlike the present study, the previous studies mentioned above did not quantify the increment or decrement in control efficacy of insecticides under two specific conditions of direct larval exposure and treatment after larval burrowing inside the bolls. A significant decrease in the pest control efficacy of the tested insecticides was observed from > 80.00% in pre-larval release treatment to < 49.00% in the treatment of post-larval release and entry inside the green bolls in the present study. Thus, the results of the present study have supported the earlier findings that insecticide use becomes relatively less useful against pink bollworms when the larvae burrow inside the green bolls (Fand et al. 2019; Peddu et al. 2020; CABI 2022). Thus, the laboratory findings from the present study provide a sound basis for insisting on timely insecticide application before larvae enter inside the green bolls for effective control of cotton pink bollworms under field conditions.

Relatively lower mortality of pink bollworm larvae exposed to NSKE 0.15% EC was compensated by its adverse effects on the developmental biology of

survivors of insecticide exposure. Our findings are in agreement with the work of Hewady et al. (2002) who reported the adverse effects of NSKE 1% EC on larval and pupal development of pink bollworms, resulting in immature deformation, and reduction in fecundity, fertility and longevity of adults. However, the larval entry inside the green bolls influenced the degree of adverse side effects on the developmental biology of individuals survived the insecticide exposure. These findings underscore the importance of timely insecticide applications before the larvae enter inside the cotton bolls for effective and economical control of pink bollworms in cotton fields.

The emamectin benzoate 5% SG showed relatively weak ovicidal activity, whereas NSKE 0.15% EC had interchangeably a weak larvicidal activity. The rest of the insecticides tested in the present have exerted dual effects, i.e., ovicidal and larvicidal actions against the pink bollworm. However, these toxic effects are mainly a transient nature, i.e., strong effects exhibited due to the use of high field doses of insecticides under laboratory conditions which are expected to eventually exhibit temporal decrease under field conditions (Ambrose et al. 2002). This further indicates that the use of insecticides like NSKE having only ovicidal action is not realistic, especially in the later cotton season during which the pink bollworm infestations are usually high. Therefore, NSKE can be a better option to use during an early crop growth window of < 60 days when the commencement of egg laying by pink bollworm moths coincides with high infestation levels of sucking pests like jassids, thrips and whiteflies in cotton. Possessing ovicidal and oviposition deterrent actions against pink bollworms is an added advantage with the use of NSKE in situations when the cotton ecosystem experiences multiple pest problems simultaneously.

Generally, the combination products are expected to perform better than their components when used at their recommended doses for pest control under field conditions (European Plant Protection Organization 2012). However, in the present study, combination products did not show expected incremental toxicity compared with their components under laboratory conditions. The plausible explanation is that high field doses were applied under laboratory conditions, where the individual components also showed efficacy on par with the combination products largely due to ephemeral toxic effects akin to those observed by Ambrose et al. (2002) in case of acetamiprid, thiodicarb, and lambda-cyhalothrin against cotton bollworm. Application of insecticides at doses lower than those used in the field would have produced expected significant differences in the bioefficacy under laboratory conditions. Thus, the results of the present study under laboratory conditions represent only the potential efficacy of tested insecticides against pink bollworms, which are primarily of an ephemeral nature. Therefore, it suggests that the results obtained under laboratory setups must be cautiously interpreted while applying in field conditions. All three combination products used in the present study have cypermethrin as one of their components, which belongs to the synthetic pyrethroid group. A window-based integrated pest management (IPM) strategy for cotton pests advocated by Indian Council of Agricultural Research-Central Institute for Cotton Research (ICAR-CICR) prohibits the use of synthetic pyrethorids before the cotton crop reaches 120 days to avoid a resurgence of sucking pests and minimize the risk of resistance development in insect pests including bollworms (Kranthi 2015; Indian Council of Agricultural Research-Central Institute for Cotton Research (ICAR-CICR) 2019). In this context, the high toxic effects exerted by solo insecticide products in present laboratory investigations make them the ideal candidates of choice for use during the initial crop growth window of 60~120 d during which the pink bollworm populations are normally in low to medium proportions. The combination products can be resorted for pink bollworm management during the later cotton season when the pest infestation is likely to be higher (Fand et al. 2021). This provides a clear insight that the right choice of insecticides for the management of cotton pink bollworms is subject to key factors like the crop growth stage and the severity of pest infestation. Therefore, cotton farmers should not always rely exclusively on the assumption of better performance of combination products.

Conclusion

The results of the present study suggest that the susceptibility of pink bollworm developmental stages to the insecticides was affected by the age of eggs and the location of the larvae, whether they are exposed or buried inside cotton bolls. Older eggs and the larvae that burrowed inside the green cotton bolls were less susceptible to insecticide treatments. As the higher efficacy of insecticides against pink bollworm egg and larval stages was largely due to their ephemeral toxic effects, the insecticides with multiple toxic effects could be the superior options for pink bollworm management. Based on the knowledge generated in the present study under laboratory conditions, a field-level management strategy could be formulated that focuses on the timings of insecticide sprays targeted at egg and larval stages of pink bollworms in cotton.

Material and methods

Maintenance of stock colony of pink bollworm

During the cotton growing season of 2020/2021, the larvae of pink bollworm were initially collected by the destructive sampling of naturally infested green bolls (Fand et al. 2019 2020) from the cotton crop (cultivar Suraj, non-Bt) raised at the experimental field of (ICAR-CICR, Nagpur, Maharashtra, India. The sampled bolls were kept inside the plastic jars (45 cm × 20 cm size) at 10 bolls per jar; the mouth of the jar was covered with a piece of white muslin cloth and secured with a rubber band. The jars were maintained in the laboratory at 27 °C ± 1 °C temperature, 65% ± 5% relative humidity, and 14 : 10 (L : D) photoperiod. The newly emerged moths from jars were provided with 10% honey solution (volume/volume fraction) to ensure longevity and fecundity (Fand et al. 2011). Subsequent generations of pink bollworms were maintained in the laboratory by rearing on detached cotton green bolls (Fand et al. 2020). Approximately 10-day-old, fresh and healthy green bolls from cotton plants (cultivar: Suraj, non-Bt) raised inside the net house were excised at the base of the pedicel using a sharp cutter. The bolls were washed gently with tap water and then shade-dried (Fand et al. 2020). The detached green bolls with cut ends of stalks sealed with molten paraffin wax, wrapped with a moist cotton wick and stalk immersed individually in Eppendorf tubes (2 mL capacity) containing 10% sucrose solution (v/v) were used for culturing the insect. The newly emerged moths (< 8 h) at two pairs per jar were caged in plastic jars (45 cm × 20 cm size), each holding 10 fresh detached green bolls. Such multiple jars were held separately, thus obtaining enough population of pink bollworms for further experiments on the evaluation of insecticide bioefficacy. The open top of the jar was covered with a piece of white muslin cloth and tied with a rubber band. Two wicks of absorbent cotton, one soaked with water and another with 10% honey solution as food for adult moths were hung inside from the wall of each jar using a thread. The jars were maintained in the laboratory at 27 °C ± 1 °C temperature and 65% ± 5% relative humidity. The moths were transferred daily to new jars holding fresh bolls throughout the oviposition period of female moths. The newly hatched larvae entered the bolls, fed and pupated within the bolls themselves. The newly formed pupae were removed and maintained till adult emergence in small plastic containers secured with lids having perforated mesh for aeration. The sex differentiation was done at the pupal stage (Fry 2014) and the male and the female pupae were kept separately till adult emergence. The newly emerged moths were again transferred in new moth mating jars to continue the cycle of oviposition on detached green bolls. The culture thus developed was used for subsequent experimentation on the bioefficacy evaluation of different tested insecticides.

Bioefficacy evaluation of insecticides

The major limitation of laboratory-based studies is the failure of bioassay data to predict field response to similar treatments. This is mainly due to the use of lower doses of test insecticides under laboratory conditions. Therefore, in the present study, the nine different insecticides were used for evaluation in the laboratory at their recommended field doses (Table 4) which are relatively higher than those used in laboratory studies. All the insecticides were selected as per the approved list of label-claim insecticides for use against pink bollworm management in cotton by the Central Insecticides Board and Registration Committee

Table 4 Insecticides evaluated for their bioefficacy against cotton pink bollworm under both laboratory and field conditions

Number	Common name and formulation	Chemical name	Recommended dose[a]		Source/ Manufacturer
			Per litre	Per ha[b]	
1	Profenophos 50% (mass fraction of active ingredient) emulsifiable concentrate (EC)	Curacron	3 mL	1 500 mL	Syngenta India Private Limited, Baner, Pune, Maharashtra
2	Lambda-cyhalothrin 5% EC	Scorpio Plus	1 mL	500 mL	FIL Industries Private Limited, New Delhi
3	Emamectin benzoate 5% soluble granule (SG)	Amnon	0.4 g	200 g	ADAMA India Private Limited, Bharuch, Gujarat
4	Ethion 50% EC	Fosmite	4 mL	2 000 mL	PI Industries Limited, Gurgaon, Haryana
5	Cypermethrin 25% EC	Superkiller	0.4 mL	200 mL	Dhanuka Agritech Limited, Gurgaon, Haryana
6	Neem seed kernel extract (NSKE) 0.15% EC	Neemstin	5 mL	2 500 mL	Ruchi oyster mushroom, Gondia, Maharashtra
7	Profenophos 40% + cypermethrin 4% EC	Profex super	2 mL	1 000 mL	NACL Industries Limited, Hyderabad, Telangana
8	Chlorpyrifos 50% + cypermethrin 5% EC	Cannon	2 mL	1 000 mL	NACL Industries Limited, Hyderabad, Telangana
9	Ethion 40% + cypermethrin 5% EC	Delmite 405	2 mL	1 000 mL	PHYTO CHEM (India) Limited, Hyderabad, Telangana

[a] According to Central Insecticides Board and Registration Committee (CIB&RC), Ministry of Agriculture, Government of India. List of label claim insecticides updated on 01/06/2023. Available online at (http://ppqs.gov.in/divisions/cib-rc/major-uses-of-pesticides)

[b] Per hectare (ha) dose is for dilution in 500 L of water

of the Ministry of Agriculture, Government of India (Directorate of Plant Protection, Quarantine and Storage 2019; 2022). The assumption behind the use of high doses of tested insecticides was that a maximum dose bioassay would more precisely measure insecticide efficacy comparable with field spray bioefficacy evaluation, and the response would be equal between the bioassay and the field spray as a measure of control accuracy for the egg and larval stages. The working solutions of insecticides were prepared in distilled water on the day of bioassay treatment and used in various experiments.

Ovicidal action

Two separate experiments were conducted, viz., one using freshly laid eggs (< 8 h old) and the other using three-day-old (~ 72 h) eggs of pink bollworm for evaluation of ovicidal action of tested insecticides. A paper strip dip method (Pineda et al. 2004; Boiteau et al. 2007) with slight modification as a spray instead of dip (Fand et al. 2009) was followed for testing the ovicidal action of insecticides. The pink bollworm eggs of respective age groups obtained from moth mating jars of the stock colony were separated from the green bolls and twigs using a camel hairbrush and collected onto plain paper. Using gum, the eggs were glued onto the strips of cardboard paper (8.0 cm × 1.5 cm) at 25 eggs per strip with the help of a fine-pointed camel hairbrush. The pink bollworm egg card strips thus prepared were sprayed individually with an aqueous solution of respective tested insecticides using a hand atomizer. Approximately 0.5 mL of the spray liquid per strip was used for spraying (Fand et al. 2009). Three replications were maintained for each insecticide treatment and thus a total of 75 eggs were evaluated for each tested insecticide. A batch of three card strips each holding 25 eggs sprayed with only water was kept as a control for comparison. The sprayed egg card strips were shade dried, labeled with experimental details like treatment and replication number, and were placed inside the glass Petri plates (15 cm diameter), separately in a batch of three replicated strips for each of the tested insecticides. The Petri plates were maintained in the laboratory at 27 °C ± 1 °C temperature and 65% ± 5% relative humidity. The post-treatment observations were recorded on the number of eggs hatched in each of the insecticide treatments at regular intervals of 24 h till the hatching was completed (Fand et al. 2009). Based on the data recorded, the percentage of egg hatching in different tested treatments was calculated by dividing the number of eggs hatched by the total number of eggs in each treatment (Fand et al. 2009). Accordingly, reduction in the egg hatching due to each insecticide treatment was computed as per the following formula (Zidan et al. 1987).

$$\textit{Reduction in hatching}\ (\%) = \left(1 - \frac{t}{c}\right) \times 100 \qquad (1)$$

where '*t*' represents the eggs hatching (%) in insecticide treatment and '*c*' represents the eggs hatching (%) in the control treatment.

Larvicidal action

Neonate larvae (< 8 h old) obtained from a pink bollworm stocky colony maintained in the laboratory were used for evaluation of the bioefficacy of tested insecticides using the boll dip bioassay technique which is a modification of leaf dip bioassay method commonly followed for the leaf-feeding insects (Perez et al. 1997; Subramanian et al. 2017). Fresh and healthy green bolls (approximately 10-day-old) were collected from the cotton plants maintained in the cage house of ICAR-CICR, Nagpur, washed with tap water to remove any inert dust and shade-dried on blotting papers. The stalks of the detached green bolls were wrapped with a moist cotton wick and were immersed individually in Eppendorf tubes (2 mL capacity) containing 10% sucrose solution to keep the bolls fresh (Fand et al. 2020).

In the first experiment, we released pink bollworm larvae onto the insecticide-pre-treated green bolls. A batch of 10 detached green bolls was dipped in a solution of each of the tested insecticides for about ten seconds and was shade-dried on the blotting paper. The insecticide-treated bolls were maintained in small plastic containers (15 cm × 10 cm) secured with lids having perforated mesh. The pink bollworm larvae were released on the treated bolls at 2 larvae per boll. The experiment was replicated thrice with a total sample size of 30 green bolls and 60 larvae being used in bioefficacy evaluation against each of the tested insecticides. In the control treatment, the neonate larvae released on the green bolls dipped into distilled water were maintained separately for comparison. The pink bollworm larvae could not sustain a prolonged period of starvation and therefore enter the green bolls within less than 5 h of hatching (Fand 2021; Fand et al. 2021). Therefore, after 24 h of exposure, the larval mortality due to the surface residue of tested insecticide applied onto the rind of green bolls was recorded in all the treatments by observing the treated bolls under a stereo zoom microscope (Model Leica, S8 APO). Besides, the number of larval entry holes if any, on the green bolls in each of the treatments was also recorded to determine the number of survivors of insecticide treatments that could enter the bolls by passing through a thin

film of insecticide residues on the surface of the bolls. The observations on the adverse effects on the developmental biology of survivors of insecticide treatments were recorded.

In the second experiment, the pink bollworm larvae were initially released on the green bolls at 2 larvae per boll with the same sample size and number of replicates as in the first experiment mentioned above. The larval entry inside the bolls was confirmed based on the number of entry holes on the boll rind observed under a stereo zoom microscope. After 24 h of entry of the larvae inside the bolls, the bolls were dipped into the solutions of tested insecticides for 10 s. A batch of green bolls dipped into only distilled water was maintained separately as a control for comparison. The treated bolls were shade-dried on the blotting paper and were maintained separately in small plastic containers (15 cm × 10 cm) secured with lids having perforated mesh in a way similar to that of the first experiment.

The treated green bolls from both the experiments described above were maintained in the laboratory at 27 °C ± 1 °C temperature and 65% ± 5% relative humidity for the next 21 days to allow the development of larvae that entered the green bolls (Fand et al. 2020). After 21 days, the number of larvae alive, if any and the pupae formed inside the green bolls from various treatments were recorded separately for the two experiments by split opening the bolls using a sharp knife. The larvae and pupae recovered from treated bolls in each of the tested insecticides were reared till their adulthood. The aberrations if any in the morphology, developmental duration and survival of immature stages, and longevity and fecundity of adults were recorded in each of the insecticide treatments.

Based on the data recorded on larval mortality in both experiments described above, the percent larval mortality in each of the insecticide treatments was computed by dividing the number of larvae that died by the total number of larvae tested (Bunga et al. 2018). Using the values of percent larval mortality in each of the insecticide treatments, the corresponding values of corrected mortality were calculated using Abbott's formula (Abbott 1925) given below.

$$\text{Corrected mortality (\%)} = \frac{\textit{Mortality in treatment (\%)} - \textit{Mortality in control (\%)}}{100 - \textit{Mortality in control (\%)}} \times 100 \quad (2)$$

Accordingly, the reduction in the insecticide efficacy to kill the larvae (%) due to larval entry inside the bolls was computed using the following formula (Proposed for the first time in the present study).

$$\textit{Reduction in insecticide efficacy to kill the larvae (\%)} = \frac{I_b - I_a}{I_b} \times 100 \quad (3)$$

where, 'I_b' represents the larval mortality recorded in insecticide treatment to the green bolls 'before' larval release and 'I_a' represents the larval mortality recorded in insecticide treatment to the green bolls 'after' release and entry of larvae inside the bolls.

Statistical analysis

The mean values were obtained from the replicated data recorded in different experiments performed. Before analysis, the arc sin transformation was applied to the percent data on egg hatching (ovicidal experiment) and larval mortality (Gomez et al. 1984). The transformed data from laboratory experiments were subjected to a one-way analysis of variance in a completely randomized design (CRD) using the Microsoft Office Excel 2010 program. The values of critical difference (CD) were computed and were used to separate the mean values at a 5% level of significance (Gomez et al. 1984). The paired *t*-test was applied to the laboratory data on egg hatching recorded in < 8 h and ~ 72 h old eggs to test if the age of the egg had any significant effect on the ovicidal action of different tested insecticides. Similarly, the effect of pre- and post-larval release treatment of insecticides on the green bolls on the efficacy of insecticide in killing the pink bollworm larvae was also evaluated by applying paired *t*-test (Student 1908). The significance of the *t*-test was adjudged based on the *P* values obtained for the respective paired treatments.

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Authors' contributions

Fand BB and Wadaskar RM planned the work, designed the experimental setup, supervised the conduct of experiments, analyzed and interpreted the data, and drafted the manuscript; Busnoor AV and Mahule DJ conducted laboratory experiments on bioefficacy evaluation, recorded the data, helped in data analysis and manuscript editing; Pillai T and Nagrare VS advised the improvements in the work plan, supervised the work progress and edited the draft of the manuscript; Tambe VJ and Prasad YG approved the work plan, provided the research facilities/ infrastructure and monitored the work progress; reviewed and edited the manuscript draft, provided insightful inputs and approved the manuscript for publication. All authors read, revised, and proved the final version of the manuscript.

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Availability of data and materials

All the data relevant to the present study are included in the article. Any further details related to the experiments conducted can be made available by requesting the corresponding author.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
The manuscript has not been published, or submitted for publication elsewhere.

Competing interests
The authors declare that they have no conflict of interest related to the content of this article.

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