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Toxicity of insecticides, fungicide, and their combinations against first instar larvae of *Spodoptera litura* (F.) on soybean, *Glycine max* (L.) Merrill

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Abstract
The present study was conducted in Insecticides toxicology laboratory, Department of Entomology, G.B. Pant University of Agriculture and Technology, Pantnagar. One fungicide, five insecticides and their combinations were tested against first instar larvae of *Spodoptera litura* and observations were recorded on percent mortality and mean leaf area consumed by larvae. Results showed that all treatments were significantly superior over control. Emamectin benzoate 5SG @ 0.002% performed best individually as it recorded highest per cent mortality (90% and 100%, mean leaf area consumed (MLAC)= 0.4 cm^2/larva and Antifeedant activity(AA)= 22.66% at 24HAE and 48HAE, respectively), it was followed by Spinetoram 11.7SC @ 0.002%, Novaluron 5.25 + Indoxacarb 4.5SC @ 0.03%, Indoxacarb 14.5SC @ 0.002% and Flubendiamide 39.35SC @ 0.01%. When these insecticides were mixed with fungicides, Fluxapyroxad 167g/l+ Pyraclostrobin 333g/l + Spinetoram 11.7SC @ (0.03 + 0.01%) showed maximum percent mortality (93.33% and 100% at 24HAE and 48HAE, respectively) and maximum reduction in feeding (MLAC= 0.23 and 0.16 cm^2/larva; Antifeedant activity= 98.64, 100, 100% at 24HAE and 48HAE, respectively) among all the treatments.

Keywords: insecticides fungicides compatibility, *Spodoptera litura, Glycine max,* leaf dip method and toxicity

Introduction
Soybean (*Glycine max* (L.) Merrill) ranks first in oilseed crop in India is known for the cheapest and highest source of edible vegetable protein among the pulse crop (Kuldeep and Ram, 2004) [4]. Brazil is leader in soybean production which is followed by USA, Argentina and China. India, ranks fourth in area with 12.12 million hectare and fifth in production with 11.23 million tonnes (Soyabean Outlook, 2022). Among the insect-pests lepidopterans are the most common affecting a wide range crops including soybean. *Spodoptera litura* Fabricius, widely known as the tobacco caterpillar, is one of the major pests of the crop, it is polyphagous in nature and cause of considerable loss of different field and vegetable crops. The larvae feed on the foliage, it completely defoliates the plant, and in cases of severe infestation, the soybean crop is completely destroyed. Out of 112 species cultivated plants which are known to be attacked by this pest, 60 are native to India and are found throughout most of Asia, including tropical, subtropical, and temperate regions, as well as Oceania (Venette *et al.*, 2003) [13]. Since chemical pesticides are frequently the first alternative that farmers in India employ, CIBRC have recommended a number of insecticides for various insects-pests. Shobharani and Kulkarni (2020) [7] examined the effectiveness of a combination of flubendiamide and hexaconazole in controlling lepidopteran pests and powdery mildew disease in blackgram. Out of the two tested dosages, the application of flubendiamide and hexaconazole at a rate of 1250 g/ha demonstrated optimal results in managing *S. litura, H. armigera,* and powdery mildew disease in blackgram, leading to an enhanced yield. The application of insecticides to control *Spodoptera* on soybean remains an essential management practise. To effectively manage *S. litura* infestations in soybean, these label-claimed pesticides must be revalidated on a regular basis. It is also critical to examine integrated pest management (IPM) practices, which emphasizes the use of diverse control approaches, such as cultural, biological, and chemical controls, to reduce pesticide reliance and environmental damage.

Materials and Methods

The experiment was conducted at Insecticides toxicology laboratory, Department of Entomology, G.B. Pant University of Agriculture and Technology, Pantnagar. The stomach toxicity of individual insecticides (5 nos.), fungicides (1 nos.), and their combinations (5 nos.) was determined against 1st instar larvae (avg. larval wt. = 0.0003g) of *S. litura* by leaf dip method under laboratory conditions (temp., 23 °C; RH= 65±2%) following Singh *et al.*, (2010) [9]; Kumar and Srivastava, (2016) [5]; Devi and Srivastava. (2018) [2]; Negi and Srivastava, (2018) [6]; and Thakur and Srivastava, (2020) [11]. The experiment was laid down in completely randomized block design (CRD) with and 11 treatments including control having 3 replications each are Emamectin Benzoate (Super yodha 1.9 EC), Flubendiamide (Flue 39.35 SC), Indoxacarb (King Doxa 14.5 SC), Spinetoram (Delegate 11.7 SC), Novaluron + Indoxacarb (Plethora 5.25+4.5% W/W SC), Fluxapyroxad 167 g/l + Pyraclostrobin (Priaxor 333 g/l), Fluxapyroxad 167 g/l + Pyraclostrobin (Priaxor 333 g/l)]2 + [Emamectin Benzoate (Super yodha 1.9 EC), Fluxapyroxad 167 g/l + Pyraclostrobin (Priaxor 333 g/l)]2 + [Flubendiamide (Flue 39.35 SC), Fluxapyroxad 167 g/l + Pyraclostrobin (Priaxor 333g/l)]2 + [Indoxacarb (King Doxa 14.5 SC), Fluxapyroxad 167 g/l + Pyraclostrobin (Priaxor 333 g/l)]2+ [Novaluron + Indoxacarb (Plethora 5.25 + 4.5% W/W SC), Fluxapyroxad 167 g/l + Pyraclostrobin (Priaxor 333 g/l)]2 + [Spinetoram (Delegate 11.7 SC) and Control.

Maintenance of host plant

The soybean seeds of the PS-1347 variety were obtained from the Department of Genetics and Plant Breeding at the University. The potted soybean plants were cultivated using agricultural methods. A total of thirty-six pots, each with a capacity of 5 liters, were utilized to accommodate twelve distinct treatments, each with three replications. Initially, five seeds were planted in each pot, but subsequently, the number was reduced to one plant per pot to ensure proper growth.

Preparation of solution

Following the recommended dosages, varying dilutions of the respective combination were created using tap water through a serial dilution technique. For combined treatments, the insecticide and fungicide were mixed in a 1:1 ratio based solely on the LC50 values (Selvaraj *et al.*, 2015). The specified doses of each individual insecticide and fungicide were mixed separately with tap water to create 50 ml volumes in conical flasks (100 ml capacity). These prepared solutions were combined equally (50 ml + 50 ml) and utilized for the experimental procedures.

The trifoliate leaves of soybean were immersed in various treatments for a duration of 3 minutes. These treatments were prepared in distinct dilutions of 100 ml, placed in glass petri plates (diameter: 9 cm), and allowed to air dry. Subsequently, each petri plate, lined with damp filter paper and holding the treated soybean trifoliate leaves, was introduced to ten larvae (with an average weight of 0.0003g). To prevent drying, the petiole of the leaves was wrapped with a moist cotton swab. The larvae were kept without food for 2 hours prior to experiment. In the control group, leaves were treated with tap water. Each treatment was replicated thrice.

The observations were recorded on per cent mortality and mean leaf area consumed (MLAC) at 24, 48, and 72 hours after feeding (HAF). The data on mortality and morbidity was corrected by Abbott's formula (Abbott, 1925) [1].

$$\text{Abbott's Corrected Mortality (\%)} = \frac{T - C}{100 - C} X100$$

Where,
T= mortality in treatments (%)
C= mortality in control (%)

MLAC Observations were taken on a graph paper and antifeedant activity (AA) was calculated by MLAC data. The data was analyzed in CRD using Duncan's Multiple Range Test (DMRT) (Duncan. 1955) [3].

$$\text{Antifeedent Activity(A.A.)} = \frac{\text{Leaf area protected in treated disc(\%)} - \text{Leaf area protected in control disc(\%)}}{100 - \text{Leaf area protected in control disc(\%)}} \times 100$$

Results and Discussion

The result of the experiment entitled 'Toxicity of insecticides, fungicide, and their combinations against first instar larvae of *Spodoptera litura* (F.) on soybean, *Glycine max* (L.) Merrill Mortality' was interpreted by taking the observations on percent mortality and mean leaf area consumed (MLAC).

Table 1: Toxicity of insecticides, fungicide, and their combinations against I instar of *Spodoptera litura* (Fab.) on soybean, *Glycine max* (L.) Merr.by leaf dip method

S. No.	Treatment Insecticide (Trade name) + Fungicide (Trade name)]	Concentration (%)	Abbott's corrected mortality (mt)%			
			24 HAE	48 HAE	60 HAE	72 HAE
1	[Emamectin Benzoate (Super Yodha 1.9 EC)]1	0.002	90.00	100.00	100.00	100.00
2	[Flubendiamide (Flue 39.35 SC)]1	0.01	0.00	23.33	63.33	73.33
3	[Indoxacarb (King Doxa 14.5 SC)]1	0.002	20.00	16.67	23.33	86.67
4	[Spinetoram (Delegate 11.7 SC)]1	0.01	86.67	100.00	100.00	100.00
5	[Novaluron + Indoxacarb (Plethora 5.25 + 4.5% W/W SC)]1	0.02	56.67	40.00	93.33	96.67
6	[Fluxapyroxad 167 g/l + Pyraclostrobin (Priaxor 333 g/l)]2	0.03	0.00	3.33	13.33	50.00
7	[Fluxapyroxad 167 g/l + Pyraclostrobin (Priaxor 333 g/l)]2 + [Emamectin Benzoate (Super Yodha 1.9 EC)]1	0.03 + 0.002	70.00	6.67	93.33	100.00
8	[Fluxapyroxad 167 g/l + Pyraclostrobin (Priaxor 333 g/l)]2 + [Flubendiamide (Flue 39.35 SC)]1	0.03 + 0.01	0.00	20.00	33.33	60.00
9	[Fluxapyroxad 167 g/l + Pyraclostrobin (Priaxor 333 g/l)]2 + [Indoxacarb (King Doxa 14.5 SC)]1	0.03+ 0.002	3.33	6.67	40.00	63.33
10	[Fluxapyroxad 167 g/l + Pyraclostrobin (Priaxor 333 g/l)]2+ [Novaluron + Indoxacarb (Plethora 5.25 + 4.5% W/W SC)]1	0.03 + 0.02	73.33	100.00	100.00	100.00
11	[Fluxapyroxad 167 g/l + Pyraclostrobin (Priaxor 333 g/l)]2 + [Spinetoram (Delegate 11.7 SC)]1	0.03 + 0.01	93.33	100.00	100.00	100.00
12	Control	C	0.00	0.00	0.00	0.00

HAE = Hours after exposure; Mean initial larval weight = 0.003 g/larva (I instar); Laboratory conditions: Temp.= 23° C, R. H.= 65% (6:00 AM); n = 10/treatment, r = 3

Table 2: Feeding toxicity of insecticides, fungicide, and their combinations against I instar of *Spodptera litura* (F.) on Soybean *Glycine max.* (L.), by leaf dip method

S. No.	Treatments [Insecticide (Trade name) + Fungicide (Trade name)]	Concentration (%)	24 HAE		48 HAE		72 HAE	
			MLAC (cm^2)	AA (%)	MLAC (cm^2)	AA (%)	MLAC (cm^2)	AA (%)
1	[Emamectin Benzoate (Super yodha 1.9 EC)][1]	0.002	0.4	22.67	0.00	100	0	100
2	[Flubendiamide (Flue 39.35 SC)][1]	0.01	0.23	54.89	0.33	42.79	0	100
3	[Indoxacarb (King Doxa 14.5 SC)][1]	0.002	0.26	48.44	0.33	36.43	0.03	45.53
4	[Spinetoram (Delegate 11.7 SC)][1]	0.01	0.33	35.56	0.00	100	0	100
5	[Novaluron + Indoxacarb (Plethora 5.25+4.5% W/W SC)] [1]	0.02	0.16	67.78	0.2	61.86	0.03	45.53
6	[Fluxapyroxad 167 g/l + Pyraclostrobin (Priaxor 333 g/l)][2]	0.03	0.13	74.22	0.36	30.07	0	100
7	[Fluxapyroxad 167 g/l + Pyraclostrobin (Priaxor 333 g/l)][2] + [Emamectin Benzoate (Super yodha 1.9 EC)][1]	0.03 + 0.002	0.2	61.33	0.13	74.57	0	100
8	[Fluxapyroxad 167 g/l + Pyraclostrobin (Priaxor 333 g/l)][2] + [Flubendiamide (Flue 39.35 SC)][1]	0.03 + 0.01	0.2	61.33	0.26	49.14	0	100
9	[Fluxapyroxad 167 g/l + Pyraclostrobin (Priaxor 333g/l)][2] + [Indoxacarb (King Doxa 14.5 SC)][1]	0.03+ 0.002	0.2	61.33	0.23	55.50	0	100
10	[Fluxapyroxad 167 g/l + Pyraclostrobin (Priaxor 333 g/l)][2]+ [Novaluron + Indoxacarb (Plethora 5.25 + 4.5% W/W SC)][1]	0.03 + 0.02	0.2	61.33	0.26	49.14	0	100
11	[Fluxapyroxad 167 g/l + Pyraclostrobin (Priaxor 333 g/l)][2] + [Spinetoram (Delegate 11.7 SC)][1]	0.03 + 0.01	0.23	54.89	0.16	68.21	0	100
12	Control	C	0.52	0	0.52	0	0.77	0

HAE = Hours after exposure; MLAC= mean leaf area consumed and AA: Antifeedant activity;

Concentration-mortality response

All the insecticides and their combinations with Fluxapyroxad 167 G/L + Pyraclostrobin 333 G/L (Priaxor), on 6 HAE and 12 HAE did not show mortality of larva (Table 1). Emamectin benzoate 5SG @ 0.002% showed 90% mortality on 24 HAE which was high among insecticides. individually and on 48 HAE 100% mortality was observed by the insecticide. Emamectin benzoate 5SG benzoate 5SG with Fluxapyrozad 167g/l + Pyraclostrobin 333g/l (0.03+0.002%) recorded 6.67%, 70%, 93.33%. Emamectin benzoate was followed by Spinetoram 5SG @ 0.002% (86.67% and 100% mortality at 24 HAE and 48 HAE, respectively), Novaluron 5.25 + Indoxacarb 4.5SC @ 0.03% (40%, 56%, 93.33% and 96.67% mortality at 24HAE, 48HAE, 60HAE and 72 HAE respectively), Indoxacarb 14.8SC @ 0.002% (16.77%, 20%, 23.33% and 86.67% at 24HAE, 48HAE, 60HAE and 72 HAE, respectively) and Flubendiamide 39.35SC @ 0.01% showed lowest mortality as it recorded 0%, 23.33%, 63.33% and 73.33% mortality at 24HAE, 48HAE, 60HAE and 72 HAE, respectively. When these insecticides were mixed with fungicide, the highest mortality was recorded on [Fluxapyrozad 167 g/l + Pyraclostrobin 333 g/l] + Spinetoram 11.7SC @ 0.03+0.01%, which is 93.33% at 24 HAE and 100% mortality was gained at 48 HAE. It was followed by [Fluxapyrozad 167 g/l + Pyraclostrobin 333 g/l] + Novaluron 5.25 + Indoxacarb 4.5SC @ 0.03+0.02% with 73.33% and 100% mortality at 24HAE and 84HAE, respectively, [Fluxapyrozad 167 g/l + Pyraclostrobin 333 g/l] + Emamectin benzoate 5SG @ 0.03+.0.002% with 6.77%, 70%, 93% and 100% mortality at 24 HAE, 48 HAE, 60 HAE and 72 HAE. The lowest mortality was observed in combination of [Fluxapyrozad 167 g/l + Pyraclostrobin 333 g/l] + Indoxacarb 14.8SC @ 0.03+0.002% and [Fluxapyrozad 167 g/l + Pyraclostrobin 333 g/l] + Flubendiamide 39.35SC @ 0.03+0.01% which recorded 3.33%, 6.67%, 40% and 63.33% mortality and 0%, 3.33%, 13.33% and 50% mortality at 24HAE, 48HAE, 60HAE and 72 HAE, respectively.

Feeding response

The insecticide individually and in combination with all fungicides showed significant reduction in the MLAC compared to control (Table: 2). At 24 HAE Novaluron 5.25 + Indoxacarb 4.5SC @ 0.03+0.02% recorded reduced MLAC (0.16, AA= 67.78%) among insecticides individually while in combination with Fluxapyrozad 167 g/l + Pyraclostrobin 333 g/l, Emamectin benzoate 5SG, Flubendiamide 39.35SC, Indoxacarb 14.8SC, and Novaluron 5.25 + Indoxacarb 4.5SC recorded MLAC= 0.2 cm^2 and AA=61.33% each. Emamectin benzoate 5SG and Spinetoram 11.7SC individually recorded lowest MLAC (0 cm^2/larvae) and when mixed with Fluxapyrozad 167 g/l + Pyraclostrobin 333 g/l, performance was retained at 48 HAE, as MLAC was found 0.36 cm^2/larvae (AA= 30.07%) and 0.16 cm^2/larvae (AA= 68.21%), respectively among the combination. While, all insecticides except Indoxacarb 14.8SC (MLAC= 0.03 cm^2, AA= 45.53%) and Novaluron 5.25 + Indoxacarb 4.5SC (MLAC= 0.03 cm^2, AA= 45.53%), fungicide and their combinations showed cent percent antifeedant activity with 0.03 cm^2 MLAC at 72 DAE.

Similar studied were conducted by Siddhartha *et al.* (2014) and results revealed that fungicide also have insecticidal properties and the mortality was significantly more at higher concentrations. Among the fungicide, mancozeb + carbendazim at 1875 ppm caused the highest mortality of 28.91%. Though, no similar studies have been reported in the literature. According to the literature that is currently accessible, the components of the current study, mancozeb and carbendazim, which are contact fungicides, demonstrate varied degrees of toxicity to different insects. In another experiment, the toxicity of insecticides with fungicide and individual insecticides was quantified. Uma *et al.* (2019) [12] investigated the compatibility of using the recommended insecticide chlorpyrifos, a combination of chlorpyrifos and cypermethrin, with a fungicide hexaconazole in laboratory conditions. This study aimed to assess their effectiveness against both the white stem borer and leaf rust, that typically emerge concurrently during the post-monsoon season. The laboratory trials demonstrated that these chemical combinations were physically compatible and there were no observable alterations in their capacity to combat the intended pests and diseases when applied in combination.

Conclusion

It could be concluded that [Fluxapyrozad 167 g/l + Pyraclostrobin 333 g/l] with Emamectin benzoate 5SG and Spinetoram 11.7SC are detrimental for growth and development of first instar larvae of *S. litura* by leaf dip method. All the combinations reduced the feeding significantly compared to control.

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