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# IMPACT OF PESTICIDE APPLICATION USING DRONE ON ENTOMOPATHOGENIC BACTERIAL DIVERSITY IN GROUNDNUT

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## ABSTRACT

The objective of the investigation is to study the diversity of *Bacillus thuringiensis* and actinomycetes in groundnut field during *kharif* 2022 after pesticide spray with different spray appliances *viz.,* knapsack sprayer, power sprayer and drone sprayer. Foliar application of imidacloprid at 30 days after sowing and chlorantraniliprole + hexaconazole at 55 days after sowing was done against the insect pests and foliar disease control in groundnut. The soil samples were collected at the time of sowing, 45 DAS and at harvest from the different treatments to study the impact or effect of different treatments on soil microbes population. The treatments were 1) Chemical sprayed with knapsack sprayer, 2) Chemical sprayed with power sprayer, 3) Chemical sprayed with drone spray with 75% RDP, 4) Chemical sprayed with drone spray with 100% RDP and 5) Chemical sprayed with 50% RDP, 6) Untreated control. The microbial organisms isolated and identified through standard microbial procedures for *Bacillus thuringiesis* and actinomycetes. The results indicated that, the number of CFUs obtained for the two organisms *i.e., B. thuringiensis* and actinomycetes from soil samples of different treatments at the time of sowing, 45 DAS and at harvest did not vary significantly. Hence, it can be concluded that, there was no significant influence of the drone spray, power spray and knapsack spray on population fluctuation of *B. thuringiensis a*nd actinomycetes in groundnut crop after one season of evaluation.

**KEYWORDS:** Actinomycetes, *Bacillus thuringiensis*, Drone spray, Groundnut insect pests, Isolation from soil.

## INTRODUCTION

Groundnut, *Arachis hypogaea* L. is an important oil seed and legume crop belongs to family Fabaceae. It is also known as peanut, earthnut, monkeynut and "king of oil seeds". The seeds contain 47-53 per cent oil, 18 per cent carbohydrate, 26 mg calcium, 401 mg phosphorus, 2.1 mg iron and vitamins like thiamine ($B_1$) 1.14 mg, riboflavin ($B_2$) 0.13 mg, niacin 17.2 mg per 100 gram of kernel. Groundnut is prominent source of dietary protein, lipids and can supply about 5.6 calories per gram and also provides cash income (Padgham *et al*., 1990).

In India, it is mainly grown in the Southern and Western states, Gujarat, Andhra Pradesh, Tamil Nadu, Rajasthan, Karnataka, Maharashtra and Madhya Pradesh, together occupying about 90 per cent of groundnut area. Globally, Groundnut covers 327 lakh hectares with the production of 539 lakh tonnes with the productivity of 1648 kg per hectare (FAOSTAT, 2021). India ranks first in groundnut area and is the second largest producer in the world with 101 lakh tonnes with productivity of 1863 kg per hectare in 2021-22 (agricoop.nic.in).

Groundnut is being attacked by different insect pests *viz*., leafhoppers*, Empoasca kerri*, aphid, *Aphis craccivora*, whiteflies, *Bamisia tabaci* and thrips, *Scirtothrips dorsalis* are most important causing serious damage throughout the crop growth period and losses may extend up to 22 per cent and 40 per cent, respectively. Similarly, the defoliators like *Spodoptera litura* and *Helicovera armigera* are also causing damage to 26-100 per cent.

Agricultural drones have the characteristics of high efficiency and reliable maneuverability, and could quickly respond to sudden pests and diseases and large-scale spraying needs (Lan and Chen, 2018). Moreover, the drones will not touch the crops, which could avoid the economic losses caused by mechanically crushing the crop. Use of drones in plant protection brings many advantages over manual spraying like less labour intensive, high uniformity in spraying, good droplet deposition both horizontally and vertically, spray fluid volume, time saving, energy saving and elimination of drudgery for the farmer. On other hand, the studies on impact of drone spray on the soil microbiota are not documented. Keeping this in view, the present studies were carried out during *kharif*, 2021.

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**Table 1. Table indicating different treatments with the spray methods, spray fluid volumes and doses of insecticides and fungicide during spray**

| Treatments | Type of sprayer | Water capacity (L ha$^{-1}$) | 1st spray Imidacloprid (ml ha$^{-1}$) | 2nd spray | |
|---|---|---|---|---|---|
| | | | | Chlorantraniliprole (ml ha$^{-1}$) | Hexaconazole (ml ha$^{-1}$) |
| $T_1$ | Knapsack sprayer | 500 L ha$^{-1}$ | 150 ml ha$^{-1}$ | 150 ml ha$^{-1}$ | 1000 ml ha$^{-1}$ |
| $T_2$ | Power sprayer | 250 L ha$^{-1}$ | 150 ml ha$^{-1}$ | 150 ml ha$^{-1}$ | 1000 ml ha$^{-1}$ |
| $T_3$ | Drone spraying | 25 L ha$^{-1}$ | 112.5 ml ha$^{-1}$ | 112.5 ml ha$^{-1}$ | 750 ml ha$^{-1}$ |
| $T_4$ | Drone spraying | 25 L ha$^{-1}$ | 150 ml ha$^{-1}$ | 150 ml ha$^{-1}$ | 1000 ml ha$^{-1}$ |
| $T_5$ | Drone spraying | 25 L ha$^{-1}$ | 75 ml ha$^{-1}$ | 75 ml ha$^{-1}$ | 500 ml ha$^{-1}$ |
| $T_6$ | Control | - | - | - | - |

## MATERIAL AND METHODS

### Experimental plot of groundnut field

The groundnut experimental plot with variety Dharani was laid in dryland farm, S.V. Agricultural college, Tirupati during *kharif*, 2022. Six treatments including untreated control was laid for studying the efficacy of different treatments against insect pests, natural enemies and soil microbes. The 6 treatments are $T_1$- Chemical sprayed using knapsack sprayer [imidacloprid 150 ml ha$^{-1}$ and chlorantraniliprole (150 ml ha$^{-1}$) + hexaconazole (1000 ml ha$^{-1}$) in 500 L ha$^{-1}$ water], $T_2$- Chemical sprayed using power sprayer [imidacloprid 150 ml ha$^{-1}$ and chlorantraniliprole (150 ml ha$^{-1}$) + hexaconazole (1000 ml ha$^{-1}$) in 250 L ha$^{-1}$ water], $T_3$- Chemical sprayed using drone 75% RDP [imidacloprid 112.5 ml ha$^{-1}$ and chlorantraniliprole (112.5 ml ha$^{-1}$) + hexaconazole (750 ml ha$^{-1}$) in 25 L ha$^{-1}$ water], $T_4$- Chemical sprayed using drone 100% RDP [imidacloprid 150 ml ha$^{-1}$ and chlorantraniliprole (150 ml ha$^{-1}$) + hexaconazole (1000 ml ha$^{-1}$) in 25 L ha$^{-1}$ water], $T_5$- Chemical sprayed using drone 50% RDP [imidacloprid 75 ml ha$^{-1}$ and chlorantraniliprole (75 ml ha$^{-1}$) + hexaconazole (500 ml ha$^{-1}$) in 25 L ha$^{-1}$ water], $T_6$- Untreated control. The treatmental details are presented in Table 1 (Knapsack sprayer capacity of 16 L, Power sprayer capacity of 20 L and drone capacity of 10 L.)

### Collection of Soil Samples

The soil samples were collected at the time of sowing, at 45 DAS and at the time of harvest at a depth of 10-15 cm into sterile polythene bags by using sterilized spatula and brought to laboratory for further processing. In the laboratory, 4 replications were maintained for each treatment and the number of CFU ml$^{-1}$ were recorded.

### Isolation of *Bacillus thuringiensis* from Soil Samples

The Sodium acetate selection method given by Travers *et al*. (1987) was followed for isolating *Bacillus thuringiensis* from soil samples with slight modifications. *Bacillus* like colonies were picked up after comparing with morphological characters like cream colored and have appearance of fried egg like colonies on plate and were purified by repeated four way streaking (Merdan *et al*., 2010). Colonies were smeared on microscopic slides after 18-24h of plating and tested for Gram's reaction and plated on $T_3$ medium and incubated for 48-72h for crystal and endospore production. The crystals and endospores were identified using the microbial procedures like crystal staining and endospore staining (Sharif and Alaeddinoglu, 1988). The collected soil samples were then subjected further processes to isolate *B. thuringenesis* at the dilution of $10^{-6}$ concentration. To isolate and to count CFU ml$^{-1}$ 4 replications were maintained from which the mean no. of colonies was recorded (Table 2).

**Table 2. Data regarding *Bacillus thuringenesis* population in groundnut experimental plot in different treatments**

| Colony Forming Units (CFU) $ml^{-1}$ | | | |
|---|---|---|---|
| **Treatment** | **At sowing** | **45 DAS** | **At harvest** |
| $T_1$ : Knapsack sprayer | $2.50 \times 10^6$ | $2.75 \times 10^6$ | $2.50 \times 10^6$ |
| $T_2$ : Power sprayer | $2.00 \times 10^6$ | $2.50 \times 10^6$ | $2.25 \times 10^6$ |
| $T_3$ : 75% RDP with drone | $2.25 \times 10^6$ | $2.25 \times 10^6$ | $2.50 \times 10^6$ |
| $T_4$ : 100% RDP with drone | $2.50 \times 10^6$ | $2.50 \times 10^6$ | $2.50 \times 10^6$ |
| $T_5$ : 50% RDP with drone | $2.00 \times 10^6$ | $2.50 \times 10^6$ | $2.25 \times 10^6$ |
| $T_6$ : Untreated control | $2.75 \times 10^6$ | $3.00 \times 10^6$ | $2.50 \times 10^6$ |
| **Mean** | **2.33** | **2.58** | **2.42** |
| **SD** | **0.30** | **0.26** | **0.13** |

**Isolation of actinomycetes from Soil Samples**

Isolation of actinomycetes was performed by using standard procedure given by Kumar *et al.,* (2010) from the soil sample collected from each treatment. The actinomycetes like appearance colonies were isolated after their identification. The collected soil samples were then subjected to further processes to isolate actinomycetes at the dilution of $10^{-4}$ concentration and 4 replications were maintained from which the mean no.of colonies were recorded (Table.4). The isolated strains were preserved at 4°C for two months and maintained for longer period by serial subculture. The cultures were observed for the Gram's reaction. The culture of Actinomycetes was subjected to biochemical tests *viz.,* indole test, simmon's citrate test (Seeley and Vandemark, 1981) and catalase test (Aneja, 2006) by standard procedures.

## RESULTS AND DISCUSSION

**Results of *Bacillus thuringiensis* population**

At the time of sowing, the no. of colonies obtained were in the range between 2.00 to 2.75 CFU $ml^{-1}$. The mean and SD were obtained as 2.33 and 0.30 respectively at the time of sowing. At 45 DAS, the no. of colonies recorded from the soil sample from which the colonies were isolated were in the range from 2.25 to 3.00 CFU $ml^{-1}$ with a mean and SD of 2.58 and 0.26 at 45 DAS. Similarly, at the time of harvest, the no. of colonies recorded were in the range between 2.25 to 2.50 CFU $ml^{-1}$ with a mean and SD of 2.42 and 0.13 respectively at the time of harvest. However, the no. of colonies recorded at the time of at sowing, 45DAS and at the time of harvest were 2.75 CFU $ml^{-1}$, 3.00 CFU $ml^{-1}$ and 2.50 CFU $ml^{-1}$ respectively in untreated control. The no. of colonies recorded at 3 different intervals were uniform with no difference from all the 6 treatments. All the *B. thuringiensis* colonies were positive for Gram staining, crystal staining and endospore staining.

The CFU's of *B.t* obtained from all the 6 treatments at three intervals from the groundnut field were uniform in population without any variation between the treatments including the untreated control plot in the field. Further it was observed that, there was no significant difference in CFUs among the treatments. The studies were pertaining to one season and due to this there was not much difference in CFU's among the treatments and intervals of collection.

The reason may be that the population of *B.t* may not be affected by the sprays in different treatments *i.e.,* knapsack sprayer, power sprayer and drone spray with different doses of chemical as the experiment was carried

**Table 3. Data regarding actinomycetes population in groundnut experimental plot at different intervals**

| Colony Forming Units(CFU) $ml^{-1}$ | | | |
|---|---|---|---|
| **Treatment** | **At sowing** | **45DAS** | **At harvest** |
| $T_1$ :Knapsack sprayer | $5.25 \times 10^4$ | $6.00 \times 10^4$ | $6.25 \times 10^4$ |
| $T_2$ :Power sprayer | $3.75 \times 10^4$ | $6.75 \times 10^4$ | $5.75 \times 10^4$ |
| $T_3$ :75% RDP with drone | $5.50 \times 10^4$ | $5.50 \times 10^4$ | $4.25 \times 10^4$ |
| $T_4$ :100% RDP with drone | $3.75 \times 10^4$ | $6.00 \times 10^4$ | $4.75 \times 10^4$ |
| $T_5$ :50% RDP with drone | $4.25 \times 10^4$ | $4.25 \times 10^4$ | $5.75 \times 10^4$ |
| $T_6$ :Untreated control | $3.50 \times 10^4$ | $3.75 \times 10^4$ | $5.25 \times 10^4$ |
| **Mean** | **4.38** | **5.38** | **5.17** |
| **SD** | **0.80** | **1.15** | **0.97** |

out for only one season *i.e.,* in *kharif* 2022 due to which the microbial count did not show any variation before and after spray, moreover in drone spray the spray deposition is mostly on leaf surface avoiding wastage on the soil surface in the field and the droplet size is also of very small, the spray may have not shown any drastic changes only for one spray. In knapsack and power sprayer also the microbial count have not varied at different intervals.

Similarly, *B. thuringiensis* was isolated from soil samples collected in central India and morphological, physiological characteristics were studied for their toxicity to crop pests by Agrahari *et al.* (2008). Another study was conducted by Rabha *et al.* (2017) collecting a total of 301 soil samples from Assam for colony morphology out of which, 42 isolates had characteristics similar to *B. thuringiensis* isolates and it was confirmed that 42 isolates are *B. thuringiensis*. Phase contrast microscopy showed that 37 isolates produced crystal endospore during the sporulation phase and 5 acrystalliferous isolates were also found.

Further studied have to be drone to find out the variation in population of *B.t* due to the effect of spray fluid on the soil by repeated experiments rather than observing the change in only one season.

**Results of Actinomycetes population**

At the time of sowing, the no. of colonies obtained were in the range between 3.50 to 5.50 CFU $ml^{-1}$. The mean and SD were obtained as 4.38 and 0.80 respectively at the time of sowing. At 45 DAS, the no. of colonies recorded from the soil sample were in the range from 3.75 to 6.75 CFU $ml^{-1}$. The mean and SD were obtained as 5.38 and 1.15 respectively at 45 DAS. Similarly, at the time of harvest, the no. of colonies recorded were in the range between 4.25 to 6.25 CFU $ml^{-1}$. The mean and SD were obtained as 5.17 and 0.97 respectively at the time of harvest. However, the no. of colonies recorded at the time of sowing, at 45DAS and at the time of harvest were 3.50 CFU $ml^{-1}$, 3.75 CFU $ml^{-1}$ and 5.25 CFU $ml^{-1}$ respectively in untreated control.

All the colonies obtained were identified as gram positive by Gram's reaction and the colonies were found positive for catalase test and negative for indole test and simmon's citrate test (Table 4).

Similarly, the actinomycetes population in groundnut field after the sprays in 6 different treatments have showed no significant difference in the microbial count at 3 intervals, which may be due to the reason that the population may not be affected by the spray in only

**Table 4. Indicating results of Gram's reaction and biochemical tests for actinomycetes isolated from soil**

| Treatment | Gram staining | Indole production | Catalase activity | Citrate utilisation test |
|---|---|---|---|---|
| $T_1$ : Knapsack sprayer | + | – | + | – |
| $T_2$ : Power sprayer | + | – | + | – |
| $T_3$ : 75% RDP with drone | + | – | + | – |
| $T_4$ : 100% RDP with drone | + | – | + | – |
| $T_5$ : 50% RDP with drone | + | – | + | – |
| $T_6$ : Untreated control | + | – | + | – |

season. Further studies have to be made for the variation in population of actinomycetes due to drone spary.

Present studies were in accordance with (Nasrabadi *et al*., 2013) who isolated actinomycetes from ninety seven samples collected from different soil ecosystems (forest, pasture, rain-fed and irrigated cultivated land) located in various climatic zones. The results are also in conformity with Daquioag and Penuliar (2021) who collected and isolated actinomycetes from four sampling sites and also tested for biochemical characterisation which were similar and in correspondence to the present study. Singh *et al.* (2016) also isolated actinomycetes from soil samples collected from different locations and tested for biochemical characterisation which suits the above study.

The isolated colonies obtained were uniform in all the 6 treatments for both *Bacillus thuringenesis* and actinomycetes isolated from soil. Even though the groundnut field was sprayed twice under 6 different treatments for the management of pests *i.e.,* sucking pest management with imidacloprid as first spray and for defoliators and leaf spot, chlorantraniliprole and hexaconazole respectively, there was no significant difference in the microbial population between the treatments, treated with 100% RDP with knapsack spray, 100% RDP with power sprayer, 75% RDP with drone spray, 100% RDP with drone spray and 50% RDP with drone spray along with untreated control. The soil microbes have not been affected by drone spray, the reason may be that drone spray in only one season may not have affected the population, but there may be a variation in the number of colonies obtained by using the drone for spray over a period of time, for which the further studies have to be made.

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