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Interference Effect of Two Entomopathogenic Organisms against Cowpea Aphid, Aphis craccivora Koch.

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ABSTRACT

The Cowpea aphid, Aphis craccivora Kock, is a common pest of several important ornamental plants. This work aims to investigate the toxic effect of Bacillus thuringiensis and Beauveria bassiana isolate on 2-3 days old adult A. craccivora, the effect of the bioinsecticide was strong on adult individuals. On the other hand the calculated LC50 values on newly emerged adults of aphid were 6.31 CFU/ml and 73868X106 spores/ml, respectively. This study has shown that, the fungus has a more effective than bacteria on adult aphids.

Keywords: Aphis craccivora, Bacillus thuringiensis, Beauveria bassiana and microbial control agents.

INTRODUCTION

Cowpea aphid, Aphis craccivora Koch., might be a genuine nuisance having a top to bottom host go. furthermore to cowpea, it overruns numerous different vegetables, cotton and furthermore as Shepherd - tote, lambsquarters, lettuce, pepperweed, Polygonum sp. what's more, Rumex sp. It infuses a solid poison into the plant while taking care of and, when populaces are enormous, this will trick or slaughter plants. While taking care of, this aphid delivers a significant measure of honeydew whereupon a dirty shape develops. The dark dingy shape lessens photosynthesis and prevents the plant development. Cowpea aphid transmits almost 30 plant infections including cotton waviness infection (Kennedy et al., 1962) and (Blackman and Eastop, 1984). Additionally, it transmits Peanut stripe infection (PStV) and Peanut mottle infection (PMV). Additionally, it had been recorded as a vector of transmission of the Sri Lankan eatable natural product mottle infection (Dassanayake and Hicks, 1992); Chili veinal mottle infection and pepper mottle infection (Cerkauskas, 2004).

Entomopathogenic growths were among the principal living beings utilized as microbial control operators for aphid species. The entomopathogenic organisms have numerous triumphs on the grounds that their attributes of good epizootic yet moderate activity and over reliance on an appropriate natural variables, which make them valuable after foundation. A significant number of them have moderately wide host ranges among bugs. Another bit of leeway is the way that they don't need to be ingested by the creepy crawly have yet can attack the host upon contact with the bug fingernail skin (Boucias et al., 1988).

Bacillus thuringiensis is a gram-positive spore surrounding bacterium B. t. has become the principle biopesticide since the beginning of the 1960s. Bacterial illnesses in frightening little creatures can be thoroughly designated bacteremia, septicemia, and toxemia. Bacteremia happens when the tiny life forms copy in the frightening little animal's hemolymph without the making of toxic substances. This situation occurs because of bacterial symbionts and every so often occurs with bacterial pathogens (Durasula et al., 1997). Septicemia happens most constantly with pathogenic microorganisms, which assault the hemocoel, copy, produce toxic substances, and murder the bug (Wang et al., 1993). Toxemia happens when the microorganisms are kept to the gut lumen and produce harms (Garczynski et al., 1991). The spore encircling bacilli have gotten the most thought as normal control administrators. Immense quantities of them produce proteinaceous bug explicit protoxins during sporulation.

The current examination was finished to consider the fatal effect of Bacillus thuringiensis and Beauveria bassiana on Aphis craccivora adults. In order to be used as microbial control administrators later on.

MATERIALS AND METHODS

1- Tested insect:

Mass raising of this A. craccivora was done in the labs of the Financial Entomology Unit, Plant Assurance Division, Desert Exploration Center. The bugs feed on bean plants (Vicia faba) by sucking plant juices. Amounts of pots were planted with seeds of bean. Exactly when the plant became over the earth, fake attack was cultivated by moving seriously attacked leaves to the new plants. Aphids were moved step by step from old to young plants. The settlement was kept up under exploration office conditions. Bringing was finished up in a case, various sides of which were involved wire work, the upper part was included glass, and the rest was wood.

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2- B.t. Isolation Technique:

Based on the acetate selective method described by (Smith et al., 1991), soil samples (0.5g) were added, each to 10 ml of LB broth buffered medium with 0.25M sodium acetate buffer at pH 6.8 in a sterile conical flask under aseptic conditions in a laminar flow workstation. The flask was incubated in a controlled environment incubator shaker, Edmund Bühler (TH25) operated at 300 rpm and 30°C for four hours. In this method, germination of B.t. spores was selectively inhibited by sodium acetate buffer (0.25M), while most of the undesired spore-formers germinated. Then suspensions were allowed to stand for 10 minutes; the upper layer of suspended samples were transferred to a sterile test tube with screw cap followed by heat treatment at 80°C for three minutes in a water bath. Heat treatment was made to eliminate all vegetative cells and non-sporulated soil microorganisms present in the samples. The samples were left to cool at room temperature before inoculating 1 ml of the supernatant using sterile pipettes onto agar plates and distributed over agar surface homogeneously. The plates were incubated overnight at 30°C; then random colonies of B.t. from agar plates were transferred onto T3 - plates using sterile loop. Transferred colonies were left for 2-3 days at least to allow complete sporulation and crystal formation characteristic for B.t. isolate. Careful aseptic techniques were done for investigating the germinated colonies using a laminar flow workstation. Examination of germinated colonies was done using stained smears method. The germinated colonies were fixed to clean slides and stained according to (Smirnoff 1962) stain method. For culturing the obtained isolates, the method Shake Flask Fermentation described by (Morris et al. 1996), Small quantities of B.t. can easily be recovered by the lactose-acetone co-precipitation procedure of (Dulmage et al. 1970), determine the number of bacteria that are present in the isolates described by (Dulmage1971), the total number of bacteria in the original solution was determined by counting the number of colony forming units (CFU's) and comparing them to the dilution factor. After that serial dilutions from the original bacterial suspension were obtained. Tube 1 contains 4.5 ml of water; in addition to 0.5 ml of the undiluted bacterial suspension to yield a total volume of 5.0 ml.

For each dilution, the number of colony forming units (CFU) on the plates was counted. Typically, numbers between 30 and 800 are considered to be in the range of statistically accurate data. To calculate the number of bacteria per ml of diluted sample, the following equation was used:

3- Fungus Isolation Technique:

 $4.5 \, \text{ml} + 0.5 \, \text{ml}$

The fungal isolate *Beauveria bassiana* was isolate from soil samples, By using the soil plate method (Warcup,

1950). Both light and electron microscopy were used for examination and identification of the fungal isolate according to Raper&Fennel, 1965 and Samson et al., 1995. The B. bassiana isolate was cultured on liquid medium after purification by sub- culting on potato dextrose agar (PDA) medium. One disc contain spores was cut from edge of actively growing culture and inoculated under aseptic condition in each sterilized media (adjusted at pH 6.5) of Potato dextrose broth (PDB 50 ml) medium in Erlenmeyer flask (250 ml capacity). The fungal isolate was transferred to an incubator maintaining 28 ±2°C. After 14 days of incubation period the mycelial mat of isolate was harvested, washed with distilled water for several time, the extract by refluxing in boiled methanol for 2 hours and then filtered off. The mycelial residue was reextracted again for three times. The combined filtrates were concentrated under reduced pressure at temperature not exceeding 35°C. The obtained residue was kept in refrigerator for investigation against the target insect. The filter of isolate was extracted by n- butanol. This step was repeated until complete extraction. The butanolic extract was filtered on anhydrous of sodium sulphate. Fungal suspension concentrations were adjusted by estimation on a haemocytometer (hirscmann 0.1X 0.0025 mm2).

4-Toxicity Test:

To determinate the effect of the *Bacillus* thuringiensis and *Beauveria bassiana* isolate on *A. craccivora*, assay was carried out on adult (2-3 days old). Three concentrations (2.5, 5, and 10 CFU/ml) of bacterial isolate and $(1X10^3, 1X10^5 \text{ and } 1 \times 10^7 \text{ spores} / \text{ml})$ of fungal suspension were used. Treatments and control represented with 3 replicates and each replicate consisted of ten adults of *A. craccivora*. Each replicate was sprayed with 1ml of bioagent in small plastic cages then transferred to 9 cm Petri dish. Control was treated with water. Daily mortality rates were noted and. Numbers of alive and dead adults recorded, Data were analyzed for determination of the lethal concentration (LC₅₀).

5-Concentration preparation:

Five dilutions of each biopesticides were prepared from a Median lethal concentration (LC_{50}). The determined quantity of each was mixed in water up to required volume to prepare 5, 10, 15, and 20% dilutions.

6- Statistical analysis and assessment of results:

Information got in various tests were exposed to measurable examination to assess the overall proficiency of the confines. Mortalities were rectified for the regular mortality as indicated by (Abbott's recipe, 1925).

The revised percent = (Observed %-Control %) x 100/(100-Control %)

Fixation/mortality relapse lines were drawn on probit logarithmic diagram as indicated by the technique created by (Finney, 1971). The LC₅₀ and LC₉₀ values were determined by probane program.

RESULTS AND DISCUSSION

1 - Toxic effect of B. thuringiensis isolate on Aphid:

The lethal effect of *B. thuringiensis* detach on *A. craccivora* adults was recorded. As showed up in (Table 1), insignificant degree of adult mortality was 11%, which was recorded with the most negligible attempted obsession

(2.5 CFU/ml), while the most imperative degree of adult mortality was (54%) was practiced at (10 CFU/ml), differentiated and 4% in ordinary mortality.

The effect of B. t. segregate on A. craccivora adults could be recognized dependent on the decided LC_{50} and LC_{90} values, which recorded 6.31 and 42.57 CFU/ml, exclusively (Fig 1).

Table 1. Toxic effect of *B. thuringiensis* isolate on 2-3 days old adult of *Aphis craccivora*.

| Cocn. | Mortality % after 3days | | | | | | |
|----------|-------------------------|-------|--|--|--|--|--|
| (CFU/ml) | Obs | Corr. | | | | | |
| 0 | 4 | 0 | | | | | |
| 2.5 | 11 | 7.29 | | | | | |
| 5 | 26 | 22.91 | | | | | |
| 10 | 54 | 52.08 | | | | | |

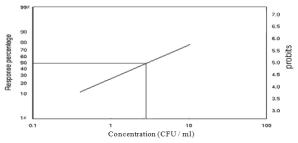


Fig. 1. Concentration / mortality regression lines for Aphis craccivora adults treated with B. thuringiensis isolate

A couple of examinations have as of late highlighted the ability of different Bacillus strains to go about as biocontrol pros against bug troubles. The effect of these organisms has been attempted equivalent to a wide extent of agronomically huge vermin including aphids (Palma et al., 2014; Rashid et al., 2017). Destructiveness of B. thuringiensis against sucker dreadful little creatures was moreover reported by (Wellman-Desbiens and Côté 2004) in an assessment coordinated on second instar pixies of the Lygus hesperus (Hemiptera: compensated with B. thuringiensis created on counterfeit eating schedule. As explained elsewhere, these frightening little creatures are seen as the most harming pros affecting a couple of yields and the cultivating economy (Leroy et al., 2011). Most examinations have separated the biopesticidal activity of the assortment Bacillus against the green peach aphid Myzus persicae (Sulzer), one of the most harming vermin, which causes colossal collect mishap the entomopathogenic bacterium B. t. (Torres-Quintero et al., 2015). This bacterium produces crystalline fuses made out of insecticidal valuable stone proteins (ICP) and endotoxins. Regardless of the way that these ICPs and toxins are significantly powerful against a couple of sets of frightening little creatures, various examinations have exhibited low to coordinate noxiousness to aphids (Porcar et al., 2009). The higher powerlessness of youthful larval instars might be either an immediate consequence of the authority of the bacterial endotoxin to the brush edge layer of the midgut epithelium (Van Rie et al., 1990) or because of certain physiological complexities between the early and late instars, where in late instars certain stimuli are created taking into account which security from the bacterial ailment might be made (Goldberg et al., 1974).

2- Toxic effect of B. bassiana isolate on Aphid:

The tried centralizations of B. bassiana detach $(1X10^3, 1X10^5, \text{ and } 1X10^7 \text{ spores/ml},)$ indicated that, deadly impacts to A. craccivora grown-ups 36, 48, and 62% individually, the mortality in all medicines was fundamentally more than in untreated (control) 4% (Table 2).

The impact of *B. bassiana* separate on *A. craccivora* grown-ups could be recognized based on the determined LC50 and LC90 values, which recorded 73868X10⁶ and 83384X10¹⁰ spores/ml, individually (Fig 2).

Table 2. Toxic effect of *B. bassiana* isolate on 2-3 days old adult of *Aphis craccivora*.

| Cocn. | Mortality % after 3days | | | | | |
|-------------------|-------------------------|-------|--|--|--|--|
| spores/ ml | Obs. | Corr. | | | | |
| 0 | 4 | 0 | | | | |
| $1X10^{3}$ | 36 | 30.8 | | | | |
| 1X10 ⁵ | 48 | 43.4 | | | | |
| 1X10 ⁷ | 62 | 57.8 | | | | |

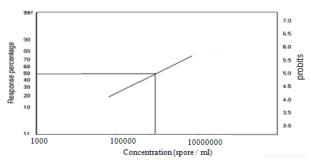


Fig. 2. Concentration / mortality regression lines for Aphis craccivora adults treated with B. bassiana isolate

are There 700 more than types of entomopathogenic growths revealed (Rabindra Ramanujam, 2007), be that as it may, a few animal groups have been viably used broadly as microbial control operators, for example, Beauveria bassiana, that assault a wide scope of vermin. The growth B. bassiana (Hyphocreales, Ascomycota) is a facultative saprophytic parasite contaminate and murder creepy crawlies and different arthropods. This organism occupies an assortment of plant animal categories as endophytes (Barelli, et al., 2016). Entomopathogenic organisms contaminate the creepy crawly through infiltration of the fingernail skin. Germ tubes develop through the layers of the fingernail skin utilizing the enzymatic activity lastly enter the haemocoel (Anderson, et al., 1995). Aphids are risky creepy crawly bugs everywhere throughout the world. Agamic generation of aphid (parthenogenesis) increment the populace mass. It is essential to welcome the impact of entomopathogenic growths on the aphid bother at various formative stages (Wang and Knudsen, 1993). As per the recorded information every single applied focus B. bassiana seclude decreased the number of inhabitants in A. craccivora grown-ups. (Griffin, 1994) expressed that the accompanying certainty; growths mystery wide exhibit of exacerbate that are naturally dynamic against different life forms. (Goettel and Jaronski, 1997) chose B. bassiana was universal as an entomopathogen of a wide scope of creepy crawlies from most bug orders. It is recorded and utilized

in stifling populace of a few monetarily significant bugs including aphids, whiteflies, mealybugs, lepidopteran eggs and vermin (Vandenberg *et al.*, 1998; and Ezz, 2004). These record deth rates might be ascribed to loss of motion (mouthpart or midgut) and/or cytotoxin impact (Maketon *et al.*, 2008).

3-Comparison between the effect of *B. thuringiensis* and *B. bassiana*, isolated on *Aphis craccivora* adults:

The main objective of current studies was to evaluate the comparative efficacy of biopesticides (*Beauveria bassiana*, and *Bacillus thuringiensis*) against Cowpea aphid, *A. craccivora*, in vitro. The study was carried out during the year of 2019, to study the effect of entomopathogenic biopesticides at different concentrations of 5%, 10%, 15% and 20% and untreated check or control. The determined quantity of each entomopathogenic biopesticides was mixed in water up to required volume to prepare dilutions/concentration. Three replications of each

treatment. Aphid was kept in ventilated plastic jars, for checking disease and parasitism, healthy individuals was used in pathogenicity assays.

The impact of various biopesticides, the mean percent mortality of aphids after the use of all focus demonstrated that the biopesticides at higher fixation (25%) and 72 hours gave the most extreme mean percent mortality, *B. bassiana* (31%), *B. thuringiensis* (23%) all treatment demonstrated the shifting level of control. As indicated by the above mean percent mortality the *B. bassiana* (half) demonstrated generally full of feeling against mustard aphid though *B. thuringiensis* 47% was least compelling appearing by the percent mortality (Table 3),. Comparative outcomes were likewise announced by (Ujjan and Shahzad 2012). (Suresh *et al.* 2012) recorded percent ethical quality with 12-hour stretch as long as seven days and presumed that mortality of aphid expanded with the expansion in

Table 3. Comparison between the effect of B. thuringiensis and B. bassiana isolated on Aphis craccivora adults:

| | Mortality % after indicated days | | | | | | | | | | | | | |
|------------|----------------------------------|----|----|---|---|-------------------|-------|-------------|---|----|---|---|-------------------|-------|
| Conc. % | B. thuringiensis | | | | | | | B. bassiana | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | Total mortality % | | 1 | | 2 | 4 | | Total mortality % | |
| | 1 | | | | | Obs. | Corr. | 1 | 2 | 3 | 4 | 3 | Obs. | Corr. |
| 0 | 0 | 2 | 2 | 0 | 0 | 4 | 0 | 0 | 2 | 2 | 0 | 0 | 4 | 0 |
| 5 | 0 | 4 | 3 | 0 | 0 | 7 | 3.12 | 0 | 0 | 6 | 1 | 1 | 8 | 4.16 |
| 10 | 2 | 4 | 15 | 2 | 0 | 23 | 19.79 | 0 | 8 | 14 | 3 | 0 | 25 | 21.87 |
| 15 | 4 | 6 | 19 | 4 | 0 | 33 | 30.20 | 5 | 7 | 24 | 1 | 0 | 37 | 34.37 |
| 20 | 8 | 10 | 23 | 6 | 0 | 47 | 44.79 | 4 | 9 | 31 | 6 | 0 | 50 | 47.92 |

Based on above conversation, it might be recommended that as well as can be expected, be utilized against cowpea aphid. On the numerical premise be that as it may, among biopesticides B. bassiana was discovered best than *B. thuringiensis*. Biopesticides can be promising and exchange candidate against compound pesticides in coordinated bug the board with less possibility of creepy crawly obstruction improvement, well being and ecological peril and useful fauna.

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تأثير تداخل اثنين من الكائنات الدقيقه الممرضه للحشرات ضد حشرة من الفول محمد إبراهيم إمام كلية العلوم - قسم علم الحشرات - جامعة عين شمس

من المعروف أن حشرة المَن من الأفات الخطيرة التي تسبب أضرار جسيمة للمحاصيل الزراعيه الاقتصادية. وفي هذة الدراسه تم أختبار تأثير سلالة Beauveria bassiana على نسب من البكتيريا الممرضة للحشرات أيضا من نوع Beauveria bassiana على نسب الموت للطور البالغ لحشرة من الفول وتحديد التركيز نصف المميت (LC50) والذي قدر (6.31 CFU/ml × 73868 spores/ml) . وقد اثبتت هذه الدراسه أن للفول تأثيرا أكثر فاعلية من البكتريا على الافراد البالغه لأفة المن .