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Combined effect of Entomopathogenic Fungus (*Beauveria bassiana*, Imidacloprid and Potassium Silicate against *Cnaphalocrocis medinalis* Guenée (Lepidoptera: Pyralidae) in rice crop

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Abstract
Present investigation was carried out to evaluate the relative efficacy of ecofriendly formulation of *Beauveriabassiana* (1.3×10 ^{6}conidia/ml), Potassium silicate (50mg/lit) and Imidacloprid (75ml/acre) for sustainable management of *Cnaphalocrocis medinalis*. During the treatments, percent mortality, population density and infestation was observed after first and second spray application at different time intervals i.e., 2*DAT, 5*DAT, 10*DAT, 15*DAT, 20*DAT. All the treatments significantly reduced population of *Cnaphalocrocismedinalis*compared to control due to their specific mode of action. Mortality was maximum (61.91%) with combined application of Potassium silicate, *B. bassiana* and Imidacloprid after 20th day of application. Similarly population density and infestation was lowest after application of Potassium silicate, *B. bassiana* and Imidacloprid after 20 days interval. The above findings showed that these combinations can be successfully integrated in future IPM programs for *Cnaphalocrocismedinalis.*

Keywords: *Beauveria bassiana,* Imidacloprid, Potassium silicate, *Cnaphalocrocis medinalis*

1. Introduction
Rice (*Oryza sativa* L.) ranks second after wheat in terms of area harvested and has important role in the economy of Pakistan [1]. There are number of factors which lead to the reduction in rice yield, among these the losses caused by insect pests varies from 20-30% annually [2, 3]. Different studies have been reported that about 128 different species of insect pests infest the rice crop but 20 among these are of major economic importance [4].`In Pakistan, stem borers, white backed plant hoppers, leaf folders and grasshoppers are the most significant pests and cause major damage to rice crop which may fluctuate from 40-50% [5, 6, 7].
Cnaphalocrocis medinalis Guenée (Lepidoptera: Pyralidae), leaffolder has attained the status of amajor pest in rice growing countries of Asia and may cause 63- 80 percent yield losses in rice [8, 9]. Neonate larvae move to the tip of leaf and feed inside the fold. Second instar larvae fold the leaf and feed inside [10]. *Cnaphalocrocis medinalis* has inflicted severe losses in paddy crop as its outbreak was epidemic in 1989 [11]. Usually, the pest causes 15-25% yield losses in basmati rice in Pakistan [12]. The losses can reach 50% if the pest is not timely controlled [13].
Beauveria (Balsamo) Vuillemin (Ascomycota: Hypocreales) is a significant natural pathogen of insects and can infect more than 700 species of arthropods [14]. These fungi infect their hosts by penetrating through the cuticle, gaining access to the haemolymph, producing toxins, and grow by utilizing nutrients present in the haemocoel to avoid insect immune responses [15]. Entomopathogenic fungi may be applied in the form of conidia or mycelium which sporulates after application [16]. Their hosts comprise of numerous pests and its large distinction in virulence towards different insect hosts makes it one of the more resourceful entomophagous fungi for the biological control of insect pests [17]. It was found that there were excellent toxic effects of *B.bassiana* and Diatomaceous earth in combination against different insect pests [18].
Imidacloprid is a systemic insecticide [19]. It acts as a nicotinic acetylcholine (Ach) agonist [20]. It binds irreversibly to the nicotinergic receptors in postsynaptic nerves, preventing acetylcholine from binding. Imidacloprid is not degraded by acetylcholinesterase [21]. This blockage leads to the accumulation of acetylcholine, which ultimately results in paralysis and death. It has a higher binding affinity for insect nerve receptors when compared to mammalian

receptors and safer to use [22]. In evaluation of insecticides including imidacloprid, it was found safe to control rice pests [23]. It was observed that use of imidacloprid did not have any negative effect on the emergence and parasitism of bio-agent *T. chilonis* [24].

The *B. bassiana* gave very promising results when used alone and in combination with imidacloprid [25]. The *B. bassiana* also showed strong synergistic effect with diquat (Herbicide) for the control of Colorado potato beetle [26]. Enhanced effects of *B. bassiana* were also observed in combination with imidacloprid against *L. decemlineata* [27].

Silicon application in paddy seedlings, improved growth and suppress pest population [28, 29, 30].Application of potassium silicate and other silicon compounds shows the suppression of insect pests such as sap suckers, borers, folivores, and non-insect pests like mites and spider [31, 32, 33]. Studies have showed that at high level of silicon, fewer planthopper becomes adults and there was decrease in female fecundity and adult longevity [34].

The efficiency of various chemical insecticides has been restricted because *C. medinalis* have developed resistance against synthetic chemicals; therefore, it was useless to apply synthetic chemicals [35, 36]. Exhaustive attempts have been made to discover alternate methods of insect pests control [37].

Over reliance and indiscriminate use of highly toxic and hazardous pesticides has resulted in pest resistance, resurgence and higher magnitude of environmental pollution leading to imbalance in natural ecosystem. Hence, the use of alternate eco-friendly strategies like microbial insecticides and use of micronutrients to induce host plant resistance, is a more appropriate approach to suppress pest population. The objective of this study was to investigate the combined effect of *Beauveria bassiana*, Imidacloprid and Potassium silicate against *Cnaphalocrocis medinalis Guenee* (Lepidoptera: Pyralidae) under normal conditions.

2. Materials and Methods

2.1. Objective

A field experiment was conducted to investigate the combined effect of *Beauveria bassiana*, Imidacloprid and Potassium silicate against *Cnaphalocrocis medinalis Guenee* (Lepidoptera: Pyralidae) under normal conditions.

2.2 Field Preparation and experimentation

Field was well prepared according to the paddy field requirements. Irrigation and fertilizer was applied constantly to meet the crop nutrients requirements adequately. There wereeighttreatments [T_1- Potassium silicate alone; T_2-*Beauveria bassiana* alone; T_3-Imidacloprid alone; T_4-*Beauveria bassiana* + Potassium silicate; T_5-Potassium silicate+ Imidacloprid; T_6-*Beauveria bassiana* + Imidacloprid; T_7-*Beauveria bassiana* + Imidacloprid + Potassium silicate and T_8-Untreated check]. Agronomic practices were followed uniformly in all the plots. The experiment was laid down in Randomized Complete Block Design (RCBD) with factorial arrangement having three replications.

Silicon treatment in the form of potassium silicate was applied immediately after transplanting rice nursery (35-40 days old). The incidence of Rice Leaf folder was observed at regular intervals in each experimental unit and pre-treatment data was recorded. When population density of Rice Leaf folder reached up to ETL level (2 freshly damaged leaves/hill), then 1stapplication of treatments was carried out. *Beauveria bassiana* and Imidacloprid was applied with the help of hand sprayer according to the field rates in order to suppress the test insect pest population.

The post treatment data regarding leaf infestation caused by *C. medinalis* was recorded after 3, 5, 7, 10, 15 and 20 days of treatments application by using standard procedures. Second application of treatments was applied after 25th day of 1st application of treatments and data was recorded after the same interval of time as in the first application.

2.3 Statistical Analysis

Standard procedures were followed to record the data. The data collected was analyzed statistically by using SPSS. The treatment means were compared by Duncan's Multiple Range Test (DMRT) for their significance at 5% probability level [38].

3. Results and Discussion

3.1 Effect on Mortality

The results revealed that all the treatments have significant insecticidal activity against *Cnaphalocrocis medinalis*, both when applied alone or in combination. The results were more protruding, when applied in combination as compared to alone.

Mortality was highest (61.91%) (Fig. 1), when Potassium silicate, *B. bassiana* and Imidacloprid were applied in combination, followed by *B. bassiana* + imidacloprid (41.55%) and that was at par with Potassium silicate + Imidacloprid (39.78%) after 20th day of treatment application of 1st spray. Mortality was 37.91% in case of Imidacloprid, followed by Potassium silicate + *B. bassiana*, *B. bassiana* and Potassium treated plots with mean mortality values of 34.68, 22.01 and 14.06%, respectively.

Similarly, after the application of 2nd spray, highest mortality was 98.42% (Fig. 2) in case of Potassium silicate + *B. bassiana* + Imidacloprid treated plots, followed by *B. bassiana* + Imidacloprid (74.57%), Potassium silicate + Imidacloprid (64.52%), Imidacloprid (62.22%), Potassium silicate + *B. bassian* (60.47%), *B. bassiana* (46.38%) and mortality was lowest (37.48%) when Potassium silicate was applied.

Furthermore, results regarding mortality (Fig, 1, 2) showed that effect of time interval was also statistically significant. Mortality values in case of Potassium silicate treated plots were 2.35, 4.46. 7.97, 10.71 and 14.06% after 2, 5, 10, 15, and 20th day of treatment application. While in case of *B. bassiana*, mortality values varried from 4.06 to 22.01% after 2nd and 20th day of treatment application. Mortality in case of Imidacloprid treated plots was in ascending order as 2ndday (9.06%) >5th day (14.89%) > 10th day (22.26%) > 15th day (30.38%) > 20thday (37.91%). Mortality values in Potassium silicate + *B. bassiana* + Imidacloprid treated plots were 17.73, 24.02, 36.19, 49.27 and 61.92%, after 2, 5, 10, 15 and 20th, respectively. Similar trend was observed after 2nd spray application.

Almost the same results were also explored by Anderson *et al.* [39]. According to his findings, higher insect mortality was observed when combined application of *B. bassiana* and sublethal concentrations of insecticides was applied against Colorado potato beetle (*Leptinotarsa decemlineata*), and also resulting into higher rates of synergism between two agents. Hassan and Charnely [40] also studied the combined applications of entomopathogens and sublethal dosages of synthetic insecticides. Synergistic and additive effects leading to increased mortality were observed by the combined application of imidacloprid and *M. anisopliae.*

3.2 Effect on Population Density

Results regarding population density of rice leaf folder showed that all the treatments have significant reduction in population, and more promising when combined application of Potassium silicate, *B. bassiana* and Imidacloprid was carried out.

Population density (Fig. 3), after 20th day of 1st spray application was lowest (5.20%) in Potassium Silicate + *B. bassiana* + Imidacloprid treatment which was statistically similar with *B. bassiana* + Imidacloprid (5.60%) and Potassium silicate + Imidacloprid (5.94%). After control treatment, population density was highest (8.06%) in potassium silicate treated plots. Population density values ranged from 5.67- 8.48% after 15th day of treatment application. Similarly, after 2nd day of treatment application, population density was maximum (11.66%) in potassium silicate and minimum (8.86%) in Potassium silicate + *B. bassiana* + Imidacloprid treated plots.

Similarly, after 2nd spray application (Fig. 4), population density of *Cnaphalocrocismedinalis* was minimum (1.43%) in Potassium silicate + *B. bassiana* + Imidacloprid treated plots, followed by *B. bassiana* + Imidacloprid (3.26%) and that was at par with Potassium silicate + Imidacloprid (3.86%). In Potassium silicate treated plots population density was highest (6.00%) after 20th day of treatment application. It was also observed that there was a decreasing trend in population density from 2ndto 20th day of treatments application.

Ferron [41] suggested almost similar findings about the combined application when he used the mycoinsecticide 'Boverin' based on *B. bassiana along* with the reduced doses of trichlorophon against the second-generation outbreaks of *Cydia pomonella* L. He observed the significant results as compared to individual applications.

3.3 Effect on Infestation (%)

Results (Fig.5) showed that the combined effect of Potassium silicate + *B. bassiana* + Imidacloprid was quite effective in reducing the infestation by larvae of *Cnaphalocrocis medinalis*. The percent infestation after 1st spray was 19.70% with combined application of Potassium silicate + *B. bassiana* + Imidaclopridfollowed by 16.07, 14.24, 11.98, and 11.06%, after 2, 5, 10, 15, and 20th day of treatment application, respectively. Infestation in case of *B. bassiana* + Imidacloprid was between 20.75+16.32% after 2nd to 20th day of application. Highest infestation was observed in Potassium silicate treated plots as 2ndday (26.98%), 5thday (25.27%), 10thday (23.52%), 15th day (21.85%) and 20th day (19.36%). Leaf infestation was minimum (2.83%) in Potassium silicate + *B. bassiana* + Imidacloprid treated plots followed by *B. bassiana* + Imidacloprid (5.39%), Potassium silicate + Imidacloprid (8.52%), Imidacloprid (10.73%), Potassium silicate + *B. bassiana* (11.85%), *B. bassiana* (12.6%) and Potassium silicate with percent infestation value 14.27% after 20th day of 2nd spray application (Fig. 6). After 2nd day of treatment application, maximum infestation was 19.35% in Potassium silicate and minimum 10.92% in Potassium silicate + *B. bassiana* + Imidacloprid treated plots. While in case of *B. bassiana* + Imidacloprid, infestation was 15.44%, followed by Potassium silicate + Imidacloprid (16.02%) and that was at par with Imidacloprid (16.52%) after 2nd day of treatment application.

The current study results revealed that when *Beauveria bassiana* and Imidacloprid along with Potassium silicate were used in combination, they significantly reduced the infestation losses and increased yield which indicated the compatibility of entomopathogenic fungi with systemic insecticides. Similar results were also found for entomopathogenic fungi with different pesticides [42, 43, 44, 45]. Because Potassium silicate and *Beauveria bassiana* alone did not give as satisfactory results as used in combinations.

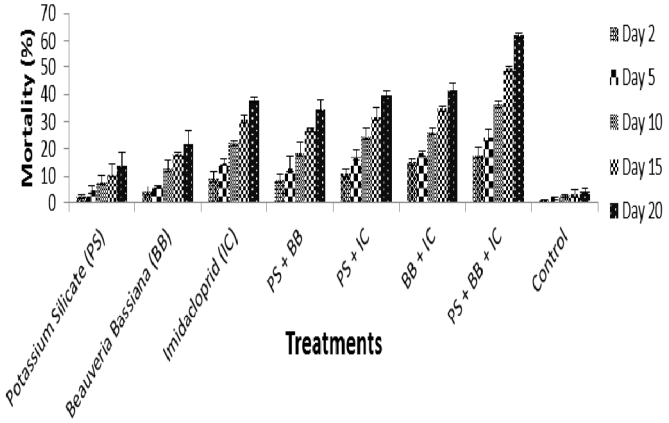

Fig 1: Effect of different treatments on mortality of *Cnaphalocrocis medinalis* after first spray at different intervals.

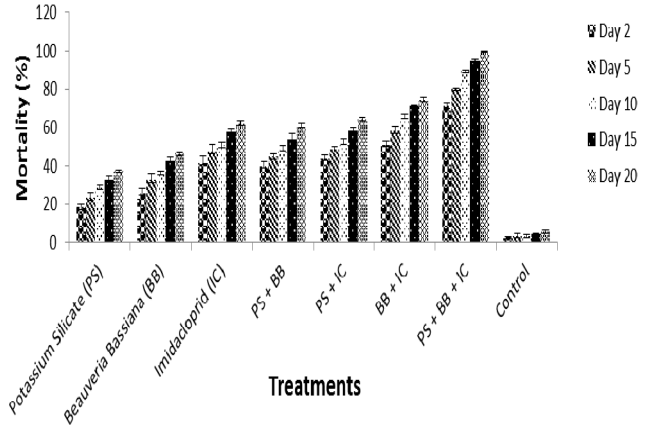

Fig 2: Comparative effect of different treatments on mortality of *Cnaphalocrocis medinalis* after second spray at different intervals.

PS= Potassium Silicate
BB=*Beauveria bassiana*
IC=Imidacloprid

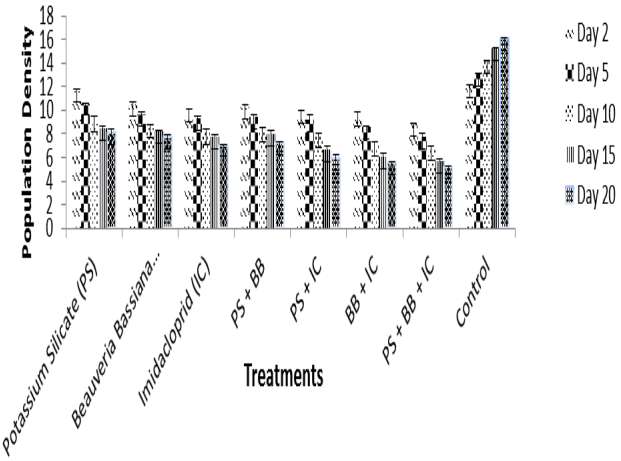

Fig 3: Comparative response of different treatments against population density of *Cnaphalocrocis medinalis* after 1st spray at different intervals.

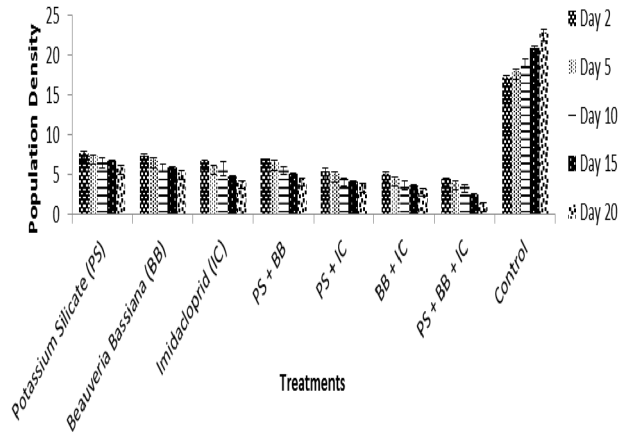

Fig 4: Comparative response of different treatments against population density of *Cnaphalocrocis medinalis* after 2nd spray at different intervals.

PS= Potassium Silicate
BB=*Beauveria bassiana*
IC=Imidacloprid

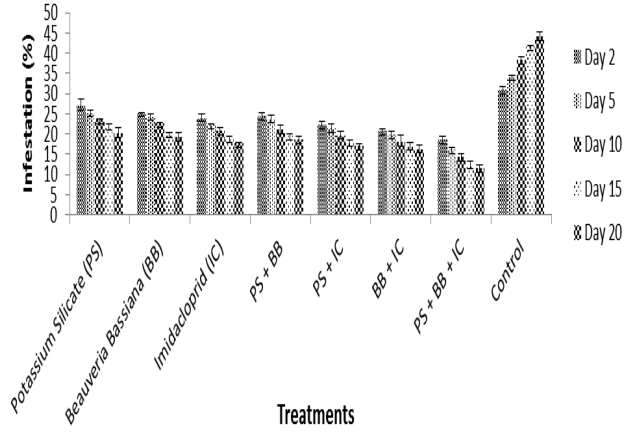

Fig 5: Comparative effectiveness of different treatment against infestation caused by *Cnaphalocrocis medinalis* at different time intervals after 1st spray application.

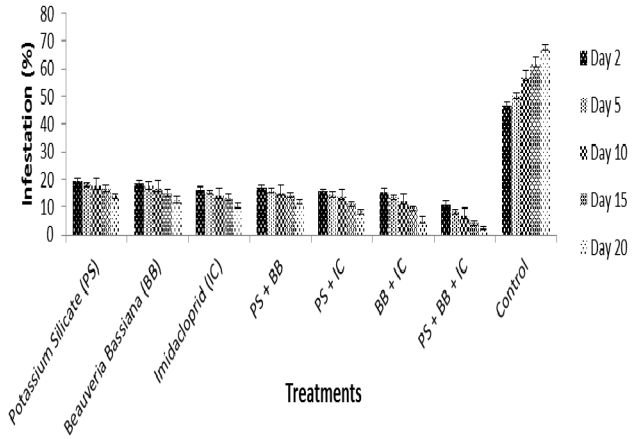

Fig 6: Comparative effectiveness of different treatment against infestation caused by *Cnaphalocrocis medinalis* at different time intervals after 2nd spray application.

PS= Potassium Silicate
BB=*Beauveria bassiana*
IC=Imidacloprid

4. Conclusion

It was concluded that maximum mortality (61.91%) of *Cnaphalocrocis medinalis* was achieved with combined application of Potassium silicate, *B. bassiana* and Imidacloprid after 20th day of application. Similarly, population density and infestation level *Cnaphalocrocis medinalis* was lowest after application of Potassium silicate, *B. bassiana* and Imidacloprid after 20 days interval. The above findings showed that these combinations can be successfully integrated in future IPM programs for *Cnaphalocrocis medinalis.*

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