Research Article

Synergistic Action of *Isaria fumosorosea* Wize (Hypocreales: Cordycipitaceae) and Spirotetramat against Asian Citrus Psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) under Field Conditions

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Abstract | Combing pesticides with different modes of action is a crucial component of pest management strategies. In this study, field efficacy of spirotetramat and *Isaria fumosorosea* formulations was assessed in spring 2016 and 2017 against Asian citrus psyllid *Diaphorina citri*, one of the economic insect pests of citrus plants and a putative vector of Huanglongbing worldwide. Treatments were comprised of label-recommended (FD) and its half (HD) dose rates of spirotetramat and *I. fumosorosea* formulations. Both treatments were applied either alone or in binary combinations. There was a significant reduction in psyllid population for all treatment combinations as compared to control ($F_{8, 107}$ = 70.36; P-value = 0.001 for 2016 and $F_{8, 107}$ = 63.58; P-value < 0.001 for 2017). Combined application of spirotetramat along with *I. fumosorosea* formulation has shown a significant synergistic effect against *D. citri* infestation and is recommended to citrus growers against sucking insect pests.

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Introduction

The genus *Citrus* is originated from the Himalayan region of Southern and North-Eastern India and adjacent China (Gmitter and Hu, 1990). Citrus fruits belong to family Rutaceae and rank first in the world both in terms of area of cultivation and production. Sweet orange, kinnow mandarin, grapefruit, lemon and lime are major citrus cultivars in Indo-Pak subcontinent (Abbas et al., 2017). However, citrus production is hampered by many species of insect pests and diseases which are detrimental for the citrus industry. Among insect pests of citrus, major ones are citrus leafminer (*Phyllocnistis citrella* Stainton), citrus psyllid (*Diaphorina citri* Kuwayama), citrus caterpillar (*Papilio demoleus* Linnaeus), citrus whitefly (*Dialeurodes citri* Ashmead), citrus mealybug (*Pseudococcid* and *Plannoccous* sp.), citrus fruit flies (*Bactrocera zonata* and *B. dorsalis* Saunders), citrus mite (*Paratetranychus citri* Mc Gregor) and citrus red scale (*Aonidiella aurantii* Maskell). Among these insect pests, Asian citrus psyllid (*D. citri*) is the most destructive pest under agro-climatic conditions of Indo-Pak region.

Asian citrus psyllid (*Diaphorina citri* Kuwayama 1907;

Hemiptera, Psyllidae) is appearing as one of the destructive sucking insect pests of citrus plants worldwide (Boykin et al., 2012). It is native to Southeast Asia (Taiwan and China) and has become an exotic pest species of citrus orchards worldwide including Australia, Brazil, India, Japan, Pakistan, South Africa and USA (Halbert and Manjunath, 2004; Yang et al., 2006; Boykin et al., 2012). Apart from their direct damage to citrus plants by sucking sap (phloem) and injecting toxic saliva, which ultimately leads to the distorted and stunted growth of young foliage, Asian citrus psyllids (ACPs) indirectly damage the citrus plants by facilitating the development of sooty mold on foliage by their honeydew secretions, and more importantly by triggering the transmission of causal agent (*Candidatus Liberibacter asiaticus*) of destructive disease of citrus *i.e.* citrus greening or Huanglongbing (HLB) (Quarles, 2013).

In general, a wide range of pesticides are being practiced to alleviate the problem of ACPs on citrus plants including organochlorides, organophosphates, carbamates, pyrethroids (Childers and Rogers, 2005; Qureshi et al., 2014). Against most of these chemical groups, ACPs have evolved certain level of field resistance (Yang et al., 2006; Tiwari et al., 2011; Hall et al., 2013). Moreover, extensive use of synthetic conventional insecticides in fruits and vegetables production is being discouraged in the current era of organic production due to potent environmental contaminations and other ecological consequences of synthetic chemical pesticides. Therefore, there is a crucial need to seek out alternate pest control strategies which could be more target-specific, safer and eco-friendly such as new-chemistry insecticides and entomopathogenic fungal formulations.

Pesticides with novel chemistry and mode of action emerge as promising options to be integrated in bio-intensive integrated pest management programs. These insecticides are more target-specific, quickly biodegradable and relatively safe to beneficial fauna including insect predators and parasitoids (Grafton-Cardwell et al., 2005; Ishaaya and Degheele, 2013; Visnupriya and Muthukrishnan, 2016), and effectively control those insect pests which have attained resistance against conventional insecticide groups (Tiwari et al., 2011; Galm and Sparks, 2016). Spirotetramat, for instance, is a novel chemistry systemic insecticide derived from spiro-cyclic tetramic acid. Regarding mode of action, it belongs to IRAC Group-23 and targets the normal activity of acetyl-CoA carboxylase of insect nervous system causing inhibition of lipid biosynthesis and normal growth (Fischer and Weiss, 2008; Nauen et al., 2008). After its first release in 2008, it has been providing excellent control of insect pests world widely, particularly of sucking pests of agricultural (Nauen et al., 2008; Brück et al., 2009; Guillén et al., 2014) and medical (Salazar-López et al., 2016) importance.

Similarly, entomopathogenic fungi such as *Beauveria bassiana, Isaria fumosorosea, Lecanicillium lecanii*, *Metarhizium anisopliae*, *Paecilomyces lilacinus* and *Pandora neoaphidis* emerge as biorational tools for controlling agricultural insect pests (Wraight et al., 2001). These naturally occurring fungi have the capability to infect and kill a wide range of insect pests including several species of chewing and sucking herbivores (Avery et al., 2013; Rios-Velasco et al., 2014). These fungi are more target specific and eco-friendly as compared to broad-spectrum synthetic pesticides. Many works have demonstrated the pathogenicity of different species of entomopathogenic fungi against mealybugs (Demirci et al., 2011; Muştu et al., 2015), whiteflies (Landa et al., 1994; Cabanillas and Jones, 2009), lepidopterous caterpillars (Xu et al., 2011; Loong et al., 2013), coleopterous grubs (Hussein et al., 2016), stored grain beetles (Riasat et al., 2013; Kavallieratos et al., 2014) etc. Recently, Stauderman et al. (2012), Conceschi et al. (2016) and Majeed et al. (2017) have shown the bioefficacy of *I. fumosorosea* and *B. bassiana* against ACPs under laboratory and field conditions.

As two of our preliminary experiments have demonstrated under laboratory bioassays that spirotetramat, a novel two-way systemic insecticide, and a commercial formulation of entomopathogenic fungus I. *fumosorosea* are the most effective biorational pesticides against ACP adults and nymphs, this field study evaluated the combined efficacy of these two pesticides against ACP populations on kinnow mandarin plants under field conditions.

Materials and Methods

The study was carried out on 4-year old citrus plants (cv. Kinnow mandarin *Citrus reticulata* var. Blanco) selected and tagged randomly in three independently selected citrus orchards in the vicinity of Postgraduate Agriculture Research Station (PARS; 31°23'N and 73°00'E) of the University of Agriculture, Faisalabad

(Punjab, Pakistan). The experiment was laid out in a randomized complete block design with 9 treatments, each with three independent replications. The experiment was repeated twice *i.e.* in the first fortnight of April 2016 and 2nd fortnight of March 2017. Two dose rates *i.e.* field-recommended (FD) and half of it (HD) were used for the commercial formulations of new-chemistry insecticide, spirotetramat (Movento® 240 SC; Bayer CropScience) and of *Isaria fumosorosea* (PFR-97® 20% WDG; formulated form naturally occurring soil bacterium Apopka strain 97 (115002) by Certis, Columbia, Maryland, USA).

Field recommended dose rates for spirotetramat and *I. fumosorosea* formulations were 96 g a.i. ha^{-1} and 900 g ha^{-1} (with *ca.* 1.0 x 10^8 colony forming units (CFU) or conidia $gram^{-1}$), respectively. Control treatment was comprised of only water which was used to prepare spray solutions for the two pesticide treatments. In each citrus orchard, nine ACP infested plants were randomly selected and tagged with red-ribbons and pest-scouting was carried out on 30 cm long apex portions of four branches, one on each of the four sides of a selected plant. ACP population, both nymphs and adults, was counted with the help of a magnifying glass carefully without disturbing the psyllids on 01 day before and 01, 03 and 07 days after treatments. Analysis of data was done using STATISTICA (V 8.1) software. After checking the normality of data via Shapiro-Wilk test, data was subjected to two-way factorial analysis of variance, taking treatment and time interval as factors, followed by Tukey's highly significant difference (HSD) test for comparison of means among the treatments.

Results and Discussion

Pesticides with a differential chemistry and mode of action than conventional ones are promising rotational tools to be integrated in different insecticide resistance management programs for the insect pests of economic importance including Asian citrus psyllids (Tiwari et al., 2011; Ishaaya and Degheele, 2013). Nevertheless, *in-situ* evaluation of tank-mix compatibility of pesticides with different modes of action has been a vital component of pest management strategies (Neves et al., 2001; Chaudhari et al., 2014; Dara, 2016). Particularly the combined applications of many insecticides and entomopathogenic fungi have been demonstrated to have synergistic action against a number of insect pests (Hiromori and Nishigaki, 2001; Irigaray et al., 2003; Farenhorst et al., 2010; Paula et al., 2011; Pelizza et al., 2015; Dara, 2016; Singh et al., 2016).

This study assessed the combined efficacy of spirotetramat, a novel insecticidal compound, and a commercial formulation of entomopathogenic fungus (*I. fumosorosea*) under field conditions against one of the destructive insect pests of citrus plants *i.e.* Asian citrus psyllid (*D. citri*). Both treatments were applied alone and in combination with two different dose rates. The experiment was conducted in April 2016 and was repeated in March 2017.

Mean population of ACP (nymphs and adults) in response to different insecticidal treatments is given in Figure 1 and Figure 2, for the year 2016 and 2017, respectively. In 2016 trial, at 01 day before treatment applications, average ACP population fluctuated from 52 to 65 ACP individuals per 4 branches without any significant difference among treatments (Figure 1). On day 01 post-treatment, all treatments except control showed ACP reduction from approximately 59 to 41 individuals per 4 branches but without any significant difference among the treatments, except for both dose rates of *I. fumosorosea* alone. Three days post-treatment, ACP reduction was significant for field dose rate of spirotetramat and for all binary combinations of insecticide and fungus, followed by insecticide half dose and both dose rates of entomopathogenic fungus alone (Figure 1). More or less similar trend has been observed in case of 7 days post-treatment.

In 2017 trial, at 01 day before treatment applications, average ACP population fluctuated from 39 to 52 ACP individuals per 4 branches without any significant difference among treatments (Figure 2). On day 01 post-treatment, binary combination of insecticide and fungus field doses showed the significant reduction of ACP population as compared to all other treatments. Three days post-treatment, ACP reduction was significant for field dose rates of the insecticide and fungus and for all their binary combinations. More or less similar trend has been observed in case of 7th day post-treatment observations; except for a significant reduction of ACP population was observed for field dose of *I. fumosorosea* (Figure 2).

On overall basis for both seasons, there was a significant reduction in the ACP population for all

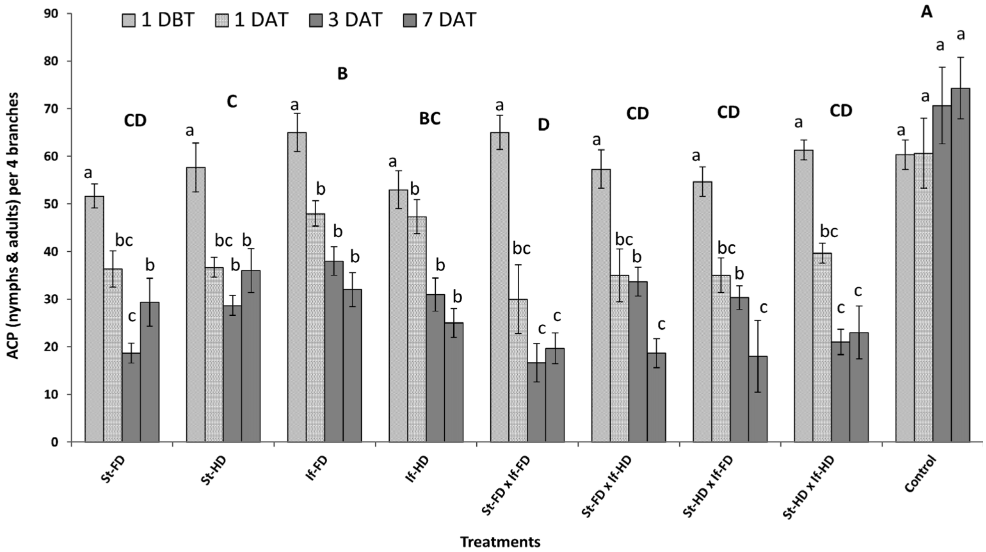

Figure 1: *Field efficacy of spirotetramat (St) and Isaria fumosorosea (If) formulations against Asian citrus psyllid (ACP) Diaphorina citri (Hemiptera: Psyllidae) in April 2016. Columns represent ACP population means ± standard error (n = 3). Different small and capital letters over bars signify statistical difference respectively among treatments for each observation time (ANOVA; P ≤ 0.05), and overall among treatments (two factor ANOVA; P ≤ 0.05).* **FD:** *field-recommended dose;* **HD:** *half of field-recommended dose.*

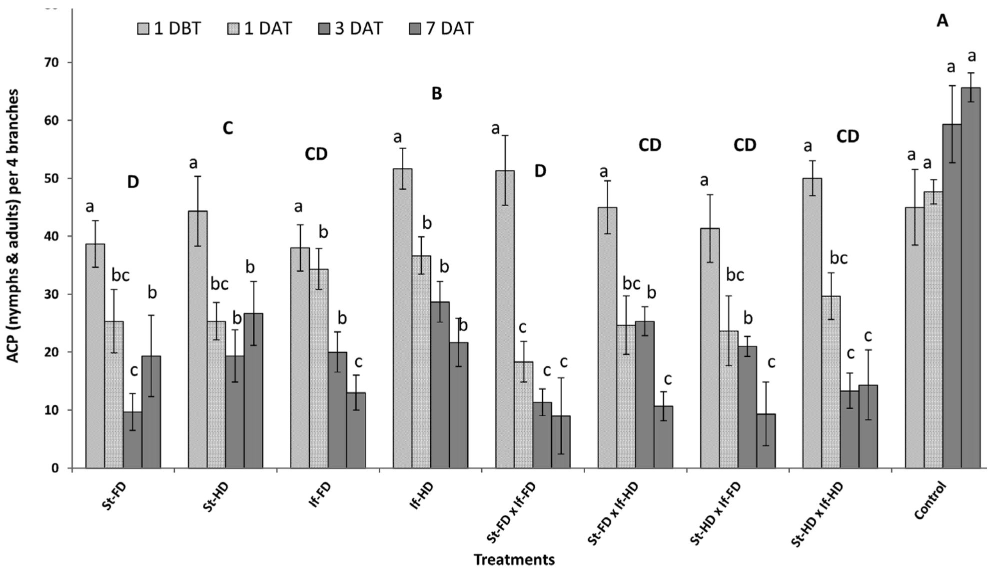

Figure 2: *Field efficacy of spirotetramat (St) and Isaria fumosorosea (If) formulations against Asian citrus psyllid (ACP) Diaphorina citri (Hemiptera: Psyllidae) in March 2017. Columns represent ACP population means ± standard error (n = 3). Different small and capital letters over bars signify statistical difference respectively among treatments for each observation time (ANOVA; P ≤ 0.05), and overall among treatments (two factor ANOVA; P ≤ 0.05).* **FD:** *field-recommended dose;* **HD:** *half of field-recommended dose.*

Table 1: *Factorial analysis of variance comparison table for the mean reduction in the population of Asian citrus psyllids (Diaphorina citri) in response to different pesticidal treatments.*

		Spring 2016		Spring 2017			
Source	DF	MS	F-value	P-value	MS	F-value	P-value
Replication	2	3.37			78.34		
Treatment	8	1319.98	70.36	0.001	1191.58	63.58	0.000
Day	3	4409.12	235.02	0.003	246.71	169.54	0.001
Treatment * Day	24	239.82	12.78	0.000	18.74	13.16	0.000
Error	70	18.76					
Total	107						
GM / CV		40.54 / 10.69					

P < 0.001 (highly significant) and P < 0.01 (significant); two-way factorial ANOVA at α = 0.05 treatments as compared to control ($F_{8, 107}$ = 70.36; *P*-value = 0.001 for 2016 and $F_{8, 107}$ = 63.58; *P*-value < 0.001 for 2017; Table 1). In 2016 trial, treatment comprising of binary combination of field doses of insecticide (spirotetramat) and entomopathogenic fungus (*I. fumosorosea*) gave the maximum and significant reduction of ACP population till the 7th day of treatment, followed by the insecticide alone and other binary combination treatments (Figure 1). In 2017 trial, again the field dose combinations of insecticide and fungus and the field dose of insecticide alone gave the significant reduction of ACP populations as compared to other treatments (Figure 2). On the contrary, there were a 23 and 46% increase of ACP population in control treatments for the year 2016 and 2017, respectively.

In general, average population of ACP remained 1.3 fold (22%) higher in 2016 than that of 2017. Most probably, this high population would be due to different experimental time periods, because under agro-climatic conditions of citrus growing areas of Indo-Pak region the population of ACP tends to build up from mid-February to early March and reaches its peak around mid-April to early May (Ahmed et al., 2004; Sharma, 2008). This seems being further corroborated by a 2-fold higher increase in ACP population in control treatments within the experimental week for 2017 (46%) than for 2016 (23%).

The average maximum reduction in ACP population for spirotetramat insecticide was observed at third day post-treatment *i.e.* about 57 and 66% respectively in 2016 and 2017 seasons. On the other hand, average maximum reduction of ACP population for *I. fumosorosea* formulation appeared on 7th day post-treatment *i.e.* about 52 and 62% respectively in 2016 and 2017 seasons. These findings are consistent with the modes of action of these pesticides because most of systemic insecticides such as spirotetramat take few hours to show their knockdown effect on target insect pests and maximum mortality occurs usually within 1–2 days post spray (Nauen et al., 2008; Simon-Delso et al., 2015), while entomopathogenic fungi usually take about 4–7 days at minimum to be active as in the field conditions these fungi require a certain time period to invade and develop their infecting mycelia on their hosts and causing host death (Diaz et al., 2006; Shahid et al., 2012; Dara, 2016; Sun et al., 2016; Majeed et al., 2017).

The overall results of these field trials demonstrated that spirotetramat is an effective and promising alternative to prevalent conventional insecticides for the management of Asian citrus psyllids and other sucking insect pests of fruits and vegetables. Moreover, combined application of spirotetramat along with *I. fumosorosea* formulation has shown a slight but significant synergistic effect against *D. citri* infestations.

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Author's Contributions

Muhammad Fiaz: performed experiments, data analysis and prepared manuscript.
Muhammad Afzal: provided technical assistance in experimentation and proof-read the manuscript.
Muhammad Zeeshan Majeed: conceived and designed the experimental protocols and performed statistical analysis.

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