Journal of Plant and Environmental Sciences

http://plantenvironment.org/

Research Article

Endophytic *Piriformospora indica* and PGPRs Implications to Enhance Tolerance in Canola Against Aphid Species

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DOI:

Received: 16/03/2022 Accepted: 21/06/2022 Published: 15/03/2024

Abstract

Canola crop is affected by many environmental factors and insect pests. Aphid is one of the major pests of canola crop and reduces the crop yield up to 70-80%. This study was conducted to assess the impact of root endophytic fungus (*Piriformospora indica*) and plant growth promoting rhizobacteria (PGPR) on aphid infestation and yield-related traits in canola crop. Three treatments (T_1: *Bacillus*; T_2: *P. indica*; T_3: *Bacillus*+*P. indica* and T_4: Water) were applied by soil application, foliar application, and soil+foliar application methods. Maximum aphid infestation was 10.11 plant^{-1} in T_3 followed T_2 (16.33 plant^{-1}), T_1 (34.22 plant^{-1}) and T_4 (106.22 plant^{-1}) in soil+foliar applications. Similar trend was recorded in foliar and soil treatments of individual and combined treatment applications. The data regarding pest infestation, plant height, yield and impact of abiotic factors were recorded from randomly tagged plants in each block of canola crop during 2021-22. The soil+foliar application technique showed significant aphid reduction followed by foliar and soil applications techniques. Maximum plant height was obtained in T_3 (*Bacillus* + *P. indica*) with an average of 99.423 cm following T_2 (94.417 cm) and T_1 (87.213 cm). The combined application (T_3) had significantly different impact on pest infestation and other yield related traits (plant height, pod length, 1000 grain weight) than individual treatments. In addition, rainfall and temperature showed negative and positive correlation with aphid infestation respectively while R.H. had not clear impact on aphid population fluctuations. Conclusively, the use of entophytic fungi and growth promoting bacteria revealed significant impact on aphid infestation reduction and yield related traits in canola crop.

Key Words: Aphid; *Bacillus*; Canola; Endophytic fungi; Abiotic factors

1. Introduction

The import of edible oil in Pakistan is around 2.421 MT at cost of 192.203 billion rupees because edible oils indigenous production meets only 25% of the total consumption in Pakistan (Minfa, 2019). Sarson (*Brassica compestris* L.), Raya (*Brassica juncea* L.) and Canola (*Brassica napus* L.) are the most widely cultivated brassica crops. *B. napus* varieties which have less than 2% erucic acid in oil are termed as canola. In rapeseed family, canola is mostly used for extraction of edible oil. Canola crop has high protein (23-25%) and oil (40-45%) contents. Oil obtaining from canola has less saturated fatty acids and more essential fatty acids which have low chances of heart problems (Barth, 2007). Among the major factors involved in declining of canola, the insect pests (aphid) are considered as serious pests (Ahmad *et al*., 2013). Aphids complete almost 45 generations in a year. Females reproduce sexually as well as parthenogenetically. Aphid infestation occurs in November and gradually increases up to March (Hashmi, 1994). Major species of aphid attacking the canola crop are mustard aphid *Lipaphis erysimi* (k.), cabbage aphid (*Brevicoryne brassicae* L.) and green peach aphid (*Myzus persicae* Sulz.). The crop losses caused by these species are almost 70-80% (Swati, 2005). Green peach aphid and cabbage aphid species are most widely distributed and abundantly found in Pakistan. They suck the cell sap and secrete honeydews which cause sooty mold infestation and affecting photosynthesis (Irshad, 2001; Emden and Harrington, 2007). The damage caused by aphids are retard plant growth, pod formation inhibition, seed oil content reduction (11%) and yield reduction upto 9-77% (Kelm *et al*., 1995). Plants failed to develop pods and also lose their vitality (Sarwar*et al*., 2011). Chemical control of aphids is only method used nowadays (Akbar *et al*., 2016). Chemical control methods impose serious threats to human beings and animals (Nawaz et al. 2014). Therefore,

To cite this paper: Ahmad, M., Ejaz, A., Munawar, N., Perveen, A., Naeem, U., Ghani, U., Mureed, M.F., Malik, A., Akbar, A., Nawaz, A. Endophytic *Piriformospora indica* and PGPRs implications to enhance tolerance in canola against aphid species. J. Plant Environ. Sci. 2023, 01, JPES07.

use of bio-pesticides is increasing due to their environment-friendly nature (Nawaz et al., 2020; 2022). Entomopathogenic fungi and different bacteria are being used for the control of different insect pests in agriculture (Nawaz et al., 2020). *Piriformospora indica* is an axenically cultivable root endosymbiont fungus which mimics the capabilities of *Arbuscular mycorrhizae* fungi. *P. indica* forms associations with rhizobacteria to promote plant growth and resistance against insect pests in a synergistic manner (Kohler *et al*., 2007). *P. indica* enables the plant to show resistance against physical and nutrient stresses and it produces resistance against metals, toxins and insects systemically (Das *et al*., 2012). Moreover *P. indica* affects the plant biomass production, induces early flowering in plants, and develops maturity in crops to escape the attack of different insect pests. In addition, PGPRs are a group of bacteria found in the rhizosphere and increase the plant growth directly or indirectly. *Bacillus*, *Azotobacter*, *Azospirillum*, *Paenobacillus* are different genera of PGPRs. Therefore, inoculation of plant rhizosphere with PGPRs results in greater development of population than normally found in the soil (Rodriguez *et al*., 2006). *Bacillus* species are abundantly found in the rhizosphere, and these have efficient abilities as PGPRs. *Bacillus* species are found in the proximal vicinity of plant roots. *Bacillus* species have broader host ranges and form biologically active compounds against targeted pests. The data regarding the combined application of bacteria and *P. indica* with different application techniques is lacking. Therefore, main objective of this study was to determine the impact of abiotic factors on efficacy of PGPR, endophytic fungus and population dynamics of aphids in canola crop production system. The study revealed quite effective outcomes to reduce aphid infestation in canola crop without using any toxic chemicals.

2. Materials and Methods

The experiment was conducted at Entomological Research Farm, near Young Wala, University of Agriculture, Faisalabad. The experimental site was between 41.47 N° latitude and 73.40 E° longitude at an elevation of 184.4 m above sea level. The canola crop was sown in October 2019. Canola variety "Super canola" was sown in blocks of 1 m^2 and treatments were applied in RCBD (Randomised Complete Block Design).

2.2 Preparation of *P. indica* inoculum

Strain of *P. indica* was obtained from Institute of Soil and Environmental Sciences (ISES) of University of Agriculture Faisalabad. Potato dextrose broth (PDB) was used to culture endophytic fungus *P. indica* (Kumar *et al*., 2011). Media was autoclaved at 121ºC for 15-20 minutes and poured in sterilized petri dishes. Incubation of petri dishes having potato dextrose broth media was done at 28±2 ºC and 65±5% R.H. and photoperiod for 12h. The conidia were collected by removing the superficial layer of two weeks old cultures. The scratched conidia were then suspended in sterile distilled water and surfactant 0.01% Tween-80 solution (Luz *et al*., 1998). The aqueous solution was then quantified using a Neubar chamber and a light microscope (Amóra *et al*., 2010). Desired concentration (1×10^8 mL^{-1}) was prepared in distilled water containing surfactant 0.1% Tween-80 and was preserved at 5 ºC until used. The inoculum was placed in agitator until used. The inoculum was used in field after 24 hours of preparation.

2.3 Preparation of PGPR inoculum

PGPRs strain (*Bacillus sp.*) was prepared in a 100 mL flask containing Trypticase Soya Agar (TSA). Around 50 mL of TSA was used in a flask to prepare the mixture. The mixture was incubated at 28±2 ºC for 2 days and was kept in a shaker at 180 rpm. Spectrophotometer was used to adjust the optical density to obtain the desired concentration (10^8-10^9 colony-forming units (CFU) mL^{-1}) of PGPRs strain. The inoculum was kept in a rotatory shaker after preparation until used.

2.4 Field bioassay study

An experiment was conducted in RCBD with block size of 1m^2. There were 4 treatments; T_1 (*Bacillus spp.)*, T_2 (*P. indica*), T_3 (combination of *P. indica* and *Bacillus spp.)* and T_4 (control) each having three replications. The concentration was $1x10^8$ cfu mL^{-1} for both *Bacillus* spp. and *P. indica.* The experimental material was applied two times with the help of hand sprinkler. First application was done after 20-25 days of crop sowing and second when crop was 50-55 days old. The treatments were applied by using three application techniques viz soil, foliar and soil +foliar application. In soil application method, material was applied within 2 inches of root zone of the crop. The pest infestation data was recorded from three randomly tagged plants from each block. Plants were tagged near the base of root above the soil surface. The data regarding aphid infestation was recorded till maturity of the crop.

2.5 Statistical analysis

The data was analyzed using the statistic version 8.1 software. One way ANOVA was applied, and means were compared using Tukey's HSD test at 5% significant level.

3. Results

Aphid appearance in canola crop was recorded from 2nd week of January to 2nd week of March and the data was also recorded during the same time period. The canola crop was infested with winged aphids to establish their population by giving birth to nymph and apterous forms. The aphid infestation started in

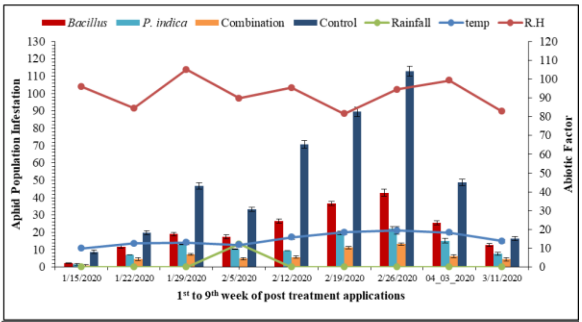

Figure 1: Population trend in foliar application method of individual and combined applications of *P. indica* and *Bacillus sp.* with abiotic factors. Control treatment showed maximum aphid infestation than applied individual and combined treatments of *Bacillus* and *P. indica*. Rainfall showed negative correlation, temperature showed positive correlation and R.H. showed no significant impact on aphid infestation on canola crop.

the mid of January and continuously increased till mid of March. There were little variations due to rainfall during the first week of February and a decline in population trend was recorded. January to February temperature range was from 10 to 19°C. The maximum and minimum relative humidity was 63 to 92% during the aphid infestation period. Relative humidity (R.H.) didn't show any significant impact on the aphid population dynamics from January to March. Maximum aphid infestation was recorded in the 4th week of February in all treatments including control treatment.

In soil application method (Fig. 1), the mean aphid population was significantly low on individual and combined application of *P. indica* with *Bacillus* as compared to control treatment. The soil application of *bacillus* alone showed maximum aphid infestation (42 aphids plant^{-1}) which was significantly high than *P. indica* (22.52 aphids plant^{-1}). The combined application (*P. indica+Bacillus*) showed lowest pest infestation (15.45 aphids plant^{-1}) than all other treatments. The minimum and maximum aphid infestation in control treatment was from 8.67 to 112.89 aphids per plant during the whole period which was always high than treated plants. The similar trend of aphid infestation was recorded in soil+foliar application of treatments but in foliar application the *Bacillus sp.* was significantly more effective than *P. indica* (Fig. 2). The mean maximum aphid infestation (16.31 aphids plant^{-1}) was recorded in the combined application of *P. indica* and *Bacillus sp* followed by individual applications of *Bacillus sp* (24.89 aphids plant^{-1}) and *P. indica* (45.67 aphids

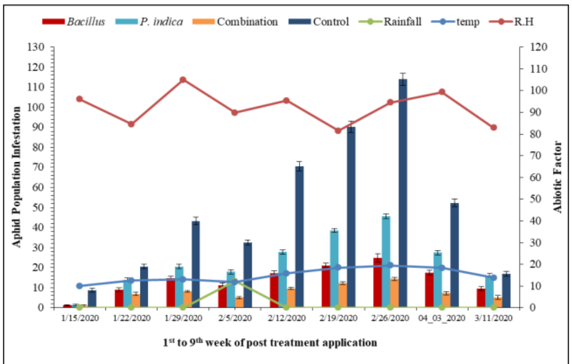

Figure 2: Population trend in foliar application method of individual and combined applications of *P. indica* and *Bacillus sp.* with abiotic factors. Control treatment showed maximum aphid infestation than applied individual and combined treatments of *Bacillus* and *P. indica*. Rainfall showed negative correlation, temperature showed positive correlation and R.H. showed no significant impact on aphid infestation on canola crop.

plant^{-1}). The aphid infestation varied from 8.67 to 113.89 aphids plant^{-1}in control which was significantly high than all other treatments. Similarly, the aphid infestation was minimum in soil+foliar application method than individual applications in soil or foliar application methods (Fig. 3). The maximum aphid population was 10.11 aphids plant^{-1} in combined application (*P. indica+Bacillus sp.*). The maximum aphid infestation in individual treatments was 16.33 aphids plant^{-1} (*P. indica*) and 34.22 aphids plant^{-1} (*Bacillus sp.*). Interestingly the individual efficacy of *P. indicia* against aphid infestation was significantly high than *Bacillus sp.* alone in soil+foliar application technique while individual techniques (soil and foliar) the use of *Bacillus sp*. was proved effective.

The tested yield parameter (plant height, pod length, 1000 grain weight) revealed significant impact of individual and combined treatments of *Bacillus sp*. and *P. indica* than control plants (Table 1). Like pest infestation results, the soil+foliar application technique proved significantly more

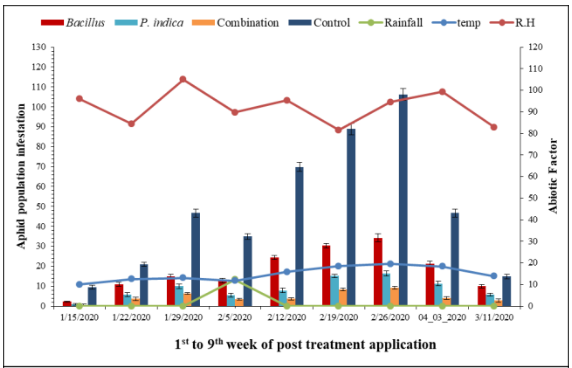

Figure 3: Population trend in foliar application method of individual and combined applications of P. indica and Bacillus sp. with abiotic factors. Control treatment showed maximum aphid infestation than applied individual and combined treatments of Bacillus and P. indica. Rainfall showed negative correlation, temperature showed positive correlation and R.H. showed no significant impact on aphid infestation on canola crop.

effective than individual applications by using soil or foliar techniques. The maximum plant height (99.42±1.67 cm), thousand grain weight (7.2±0.19 g) and pod length (6.18±0.18 cm) was recorded in combined treatments of *Bacillus sp*. and *P. indica* in soil+foliar application technique. The *Bacillus sp*. showed more impact than *P. indica* in soil application while in contrast *P. indica* produced better impact in foliar application in case of thousand grain weight and pod length. This trend was absent regarding plant height.

4. Discussion

Results of current study showed that canola crop was affected by wide variety of insect pest among all species density of *Brevicoryne brassicae*. These results were similar to study of the Ahmad *et al.* (2013) who documented that brassica species were more susceptible to the *Brevicoryne brassicae*

Table 1: Impact of individual and combined applications of *Bacillus sp.* and *P. indica* on different yield parameters of canola crop.

Biological traits	Treatment Application Method	*Bacillu ssp.*	*P. indica*	*Bacillus+ P. indica*	Control
Plant height (cm)	Soil	76.03 ± 1.17^{C}	85.8 ± 1.23^{B}	92.03 ± 1.45^{A}	66.52 ± 1.01^{D}
	Foliar	82.53 ± 1.29^{B}	73.61 ± 1.14^{C}	88.82 ± 1.45^{A}	65.25 ± 1.12^{D}
	Soil+Foliar	87.21 ± 1.36^{C}	94.45 ± 1.53^{B}	99.42 ± 1.67^{A}	70.5 ± 1.28^{D}
1000 grain weight (g)	Soil	6.46 ± 0.14^{B}	5.78 ± 0.13^{C}	6.98 ± 0.21^{A}	3.83 ± 0.12^{D}
	Foliar	4.76 ± 0.13^{C}	5.05 ± 0.18^{B}	6.52 ± 0.19^{A}	3.62 ± 0.11^{D}
	Soil+Foliar	6.94 ± 0.15^{A}	5.9 ± 0.14^{B}	7.2 ± 0.19^{A}	4.1 ± 0.11^{C}
Pod length (cm)	Soil	5.26 ± 0.09^{B}	5.06 ± 0.12^{C}	6.06 ± 0.23^{A}	3.86 ± 0.17^{D}
	Foliar	4.94 ± 0.2^{A}	5.16 ± 0.18^{A}	5.83 ± 0.11^{A}	3.75 ± 0.14^{A}
	Soil+Foliar	5.45 ± 0.21^{B}	5.29 ± 0.15^{C}	6.18 ± 0.18^{A}	3.85 ± 0.12^{D}

population. The aphid infestation was started in the 2nd week of January after almost 55 days of sowing. Ansari *et al*. (2007) also recorded an initial infestation of aphids after 60 days of sowing. The current findings revealed maximum pest infestation in the last week of February. A similar trend was also reported as a maximum number of aphid species was observed in February to end of March (Ansari *et al*., 2007). On the contrary, the number of aphids reduced up to mid of March due to changes in temperature or crop maturity.

The entomopathogenic fungi also exert dual impact on pest infestation by causing mortality or making the plant less susceptible to the insect pests (Sajid *et al*., 2017). The individual applications of *P. indica* revealed a significant impact of aphid infestation reduction in all application techniques than control. Maximum aphid infestation was 22.22, 45.67 and16.33 aphid/plant in soil, foliar, soil+foliar application treatments respectively (Fig.1-3). It has been reported that *P. indica* is an axenically cultivable root endosymbiont fungus that mimics the capabilities of *Arbuscular mycorrhizae* fungi. It mobilizes the insoluble phosphates and then transfers the phosphate to the host as energy resources (Kohler et al., 2007). The foliar application showed more infestation than other application techniques which may be due least colonization through foliar application of *P. indica*.

Bacillus species have symbiotic relationships with various plant species. It has been reported that *Bacillus subtilis*, *Bacillus cereus*, *Bacillus amyloliquefaciens* were effective against aphid and also non-toxic for natural enemies of insect pests (Gadhave *et al*., 2016). *Bacillus*, *Azotobacter*, *Azospirillum*, *Paenobacillus* are different genera of PGPRs. They are a group of bacteria found in rhizosphere and increase the plant growth directly or indirectly. Microbial inoculants of PGPRs can be used as biostimulators, bioremediators, biocontrol agents and biofertilizers. *Bacillus* species are abundantly found in rhizosphere, and these have efficient abilities as PGPRs. The *Bacillus sp.* revealed maximum aphid infestation of about 42.78, 24.89, and 34.22 aphid/plant in soil, foliar, soil+foliar application treatments respectively (Fig 1-3) which was significantly lower than control treatment. This may be because PGPRs produce secondary metabolites such as phytohormones, hydrogen cyanide, antibiotics, siderophores and volatile compounds.

In contrast to *P. indica*, *Bacillus sp.* proved effective through foliar application technique than soil application. *P. indica* forms synergistic associations with rhizobacteria to promote plant growth and resistance against insect pests (Kohler *et al*., 2007) which is also evident from the current results. The combined application (*P. indica+Bacillus sp.*) revealed maximum aphid infestation of about 13.24, 14.36 and 10.11 aphid/plant than control 112.89, 113.89, 106.22 aphids plant^{-1} in soil, foliar, soil+foliar application treatments respectively (Fig 1-3) during the whole period. *P. indica* enables the plant to show resistance against physical and nutrient stresses and it also produces resistance against metals, toxins and insects systemically (Das *et al*., 2012). Thus *P. indica* and *Bacillus sp.* provided 89% reduction in pest population which is quite consistent with previous findings of Bajaj *et al*. (2015) as they reported around 83% population reduction.

The impact of all treatments on the yield parameters of the canola crop was also significant than control treatments as reported in previous studies (Gill *et al*., 2016) that *P. indica* increased biomass production and plant height in barley crop. The combined application (*P. indica+ Bacillus*) was more effective than the other control methods used in current investigations. The maximum 1000 grain weight (7.222 g), pod length (6.18 cm) and plant height (99 cm) were obtained in combined treatments which were significantly higher that control treatment. Among abiotic factors rainfall and temperature was most important affecting the aphid population density. The effect of abiotic factors showed an increase in aphid population with an increase in temperature. The aphid population was 2.22 aphid plant^{-1} to 113.89 plant^{-1} at minimum temperature (10 ºC) and maximum temperature (19.5 ºC) respectively. The highest aphid population was observed in the 1st week of March. Akbar *et al.* (2019) reported the highest aphid population (96%) during mid of March but in current investigations aphid population was maximum in the last week of February and the start of March. The relative humidity was unable to affect pest population fluctuations in current investigations.

4. Conclusions

Conclusively, the results revealed that the combination of *P. indica* and *Bacillus sp* was more effective to control the pest population at different stages of crop than their individual applications. In individual application, *P. indica* produces most promising results in soil application technique while *Bacillus sp*. was more effective in foliar application. Among abiotic factors, temperature has positive correlation while rainfall showed negative correlation with aphid infestation on canola crop. Among application techniques, soil+foliar application technique was significantly effective than individual soil and foliar application techniques. Overall, the consortium of *P. indica* and *Bacillus sp*. produced effective against aphid infestation and yield related traits.

Acknowledgements

We would like to acknowledge Higher Education Commission (HEC) Pakistan for providing funding under NRPU 4673 project about pesticide residues and alternative solutions.

Author Contributions

MA and NM: conducted the experiment. AE: wrote the final draft. AP and UN: helped in media preparations. UG and MF: did the data collection and manipulation. AM and SA: helped in final draft preparation. AN: Reviewed and edited the final draft. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

There is no conflict of interest among authors.

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