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Review

Tomato leafminer [(*Tuta absoluta* Meyrick) (Lepidoptera: Gelechiidae)] and its current ecofriendly management strategies: A review

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Continue climatic changes in the world enhance the emergence of invasive agricultural insect pests. *Tuta absoluta* is one of the most devastating tomato leafminer and it spreads extensively in almost all parts of the world. It is a serious threat for tomato production and it results in highest damage. *T. absoluta* has fast growth rate with developmental stages of egg, larvae, pupa, and adult and it adapts to different environmental conditions. A larva is the most destructive one that consumes preferably leaves, stems and fruits of the tomato plant by hiding within mesophyll of the plant tissues. Although different environmentally hazardous pesticides are used against *T. absoluta*, none of the insecticides give full control due to the resistance development. This problem calls an urgent search for eco-friendly alternatives to control this invasive insect. Recent investigations are focused on different environmental friendly approaches that inclusively termed as IPM strategies. These are cultural practices, biopesticides, and biological using parasitoids, predators, microbial cells, microbial products, RNAi, inheritance sterility development in insect and pest resistant plant cultivar production are considered as promising alternatives to control *T. absoluta*. Therefore, this review briefly describes the current status of these methods used to design suitable and sustainable management strategies against *T. absoluta*.

Key words: Tomato leafminer, insecticide resistances, biopesticides, biological control.

INTRODUCTION

Alarmingly increasing climatic changes in the world expands the opportunity for the emergence of different invasive insect pests (Robinet and Roques, 2010). This is currently a serious threat to the agricultural industry. The insect [*Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae)] is a worldwide (Desneux et al., 2010) devastating Solanaceous plant pest. It is first discovered from Peru in 1917 and recognized as leafminer of tomato (*Solanum lycopersicum* L.) in South America in 1960 (Guedes and Picaco, 2012). The notorious manifestation of *T. absoluta*

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on tomato was detected from Spain in 2006 (Urbaneja et al*.*, 2007). This insect has alarmingly spread into Asian countries, Europeans, Mediterranean shores and recently it is also extending into African countries (Karadjova et al., 2013; Zekeya et al., 2016).

In African, *T. absoluta* was firstly reported in Algeria, Morocco, and Libya in 2008 and 2009 (Harbi et al., 2012) and continued to invade Egypt in 2010 (Moussa et al., 2013), then reached Sudan and South Sudan in 2011 (Pfeiffer et al., 2013; Brevault et al., 2014). The pest had accessed to Ethiopia in 2012 most probably from Sudan or Yemen (Goftishu et al., 2014) and Kenya in 2013 (Mohamed et al., 2015)*,* Tanzania and Senegal in 2014 (Biondi et al., 2015; Tonnang et al., 2015), Uganda in 2015 (Tumuhaise et al., 2016), South Africa in 2016 (Visser et al., 2017). The main spread vehicle for this invasive insect species was natural dispersal means, such as wind (Gontijo et al., 2013; Sridhar et al., 2014) and human fruit and vegetable translocation processes in local and abroad markets (Karadjova et al., 2013).

The possible entry site for *T. absoluta* into Ethiopia was expected from nearby Yemen, where severe tomato leafminer infestations was recorded (Gray et al., 2013) and the border of Sudan through Humera (Retta and Berhe, 2015) in the Northern part of the country. The spread of this pest was continued into Eastern (Somali and Oromia) regions and parts of shewa, then invaded the Central Refit Valley (Fortune, 2013). The distribution of *T. absoluta* in Ethiopia was terribly occupying the main tomato farming locations. Similarly, the abundance is also high when detected from moth captured using pheromone trapping method, ranging from 27 to 47 in open field and 103 to 255 in the greenhouse (Goftishu et al., 2014).

The reproduction potential of this insect is also high with a life cycle that is completed within 30 to 35 days based on the environmental conditions (Harizanova et al., 2009). Such fast reproductive character of the insect makes it more dangerous and notorious in tomato farming system.

The insect is time selective on their activity mostly concentrated in the early morning and dusk, however, in the daytime, they remain hidden among the leaves of the plant (Arnó and Gabarra, 2011). The lifespan of adult insect ranged between 10 and 15 days for females and 6 and 7 days for males. The female moth lays separately dispersed eggs on the underside of leaves especially at the apical shoot (Cherif et al., 2013), stems and sepals to facilitate fast pest distribution. The egg size is small (0.35 mm long) and each moth usually lays 250 to 300 eggs and the insect exhibits 10 to 12 generations in a year (Desneux et al., 2010). The larva is the feeding stage of this pest and can cause 80 to 100% losses in tomato if it is not controlled.

The adaptation ability of *T. absoluta* was also mostly fitted with different environmental conditions. For instance, Van Damme et al. (2015) tested the cold hardiness and overwintering potential of insect to detect the supercooling point of larvae, pupae, and adults for a lower lethal time at 0 and 5°C, reported diapause incidence at 18 and 25°C. Then they determined the mean supercooling point for pupae (-16.7°C) that was higher than for adults (-17.8°C) and larvae (-18.2°C) in which the most agro-ecology is not situated in such condition. *T. absoluta* also adapts the temperature higher than 49°C in summer as findings showed in Sudan (Tonnang et al*.,* 2015) and able to tolerate hot and dry conditions.

T. absoluta is not easily manageable by using chemical pesticides due to two reasons. First the larval stage completes its development inside the leaves mesophyll tissue. Therefore, larvae are not easily exposed to chemical insecticides (Ayalew, 2015). The second challenging thing is the resistance ability of insects towards different pesticides used (Cuthbertson et al., 2013; Ponti et al., 2015). Such pesticide resistance in insect enforces the farmer to try a number of inappropriate chemicals through spraying on their tomato farm. In fact, different types of pesticides have been used for *T. absoluta,* but none of them are significantly successful to control it (Abbes and Chermiti, 2012; Guedes and Picanço, 2012; Guedes and Siqueira, 2013). This continuous application of inappropriate agrochemicals for trial on the farmland has been aggravating residual accumulation in the environment and imposing human health problems (Abdel-Raheem et al., 2015).

Thus, to overcome such complicated problems, different findings are conducted to develop environmental friendly Integrated Pest Management (IPM) strategies. This includes mass trapping of male moths by using sex pheromone (Braham, 2014), using of phytochemicals, using biological agents such as parasitoids, predators, microbial cells, microbial products, small RNA interference (RNAi), inheritance sterility (IS) development in insect and pest resistant plant cultivar production approaches were applied to control *T. absoluta* (Van Lenteren and Bueno, 2003; Abbes et al., 2012; Cagnotti et al*.,* 2012; Camargo et al., 2016). Thus, the main purpose of this review was to compile important findings conducted on eco-friendly control of *T. absoluta* for sustainable healthy farming system.

HOST PLANT SELECTION MECHANISM AND LIFE CYCLE OF *T. ABSOLUTA*

Several insect pests use volatile attractant secretions that are emitted from the plant for their feeding and oviposition during host selection (McCormick et al., 2012; Beyaert and Hilker, 2014; Bawin et al., 2015). *T. absoluta* has such behavior to select their host plants (Megido et al., 2014) with a high preference towards tomato cultivars (Proffit et al., 2011; Shehata et al., 2016) and mostly underside of leaves for their oviposition (Cherif et al., 2013). Although tomato is the main host for *T. absoluta*, the other solanaceous plant species also served as alternative hosts.

Mostly, mated females of *T. absoluta* prefer leaves especially apex part for oviposition than the other parts of the tomato plant (Torres et al., 2001; Leite et al., 2004; Proffit et al., 2011; Cherif et al., 2013). This oviposition site preference could be associated with a lower calcium content of apical plant leaves than that of middle and basal leaves of tomato (Leite et al., 1999). The oviposition of *T. absoluta* was mostly mediated by volatile signals generated from their host plant (Proffit et al., 2011). Tomato leaf has volatile compounds such as terpenoid that attracts mated females for oviposition. Plant cultivars, rich in 2-tridecane or zingiberene that are chemicals used to give tomato resistance against beet armyworm are not preferred for oviposition by *T. absoluta* (De Oliveira et al., 2012). However, plants with the absence or less content of terpenes that is herbivores repellent compound are preferable to *T. absoluta* oviposition (Proffit et al., 2011; Shehata et al., 2016). The oviposition site selection is not only determined by plant volatile chemical attractants but also their internal physiological changes during egg laying stage such as ovarian maturation, egg production, and mating status can influence the response of the insect to host signaling (Masante-Roca et al., 2007).

Tomato plants bearing *T. absoluta* eggs produce hexanal and (Z)-3-Hexen-1-on that is secreted in response to plant damage (Anastasaki et al., 2015). Because, the chemical compound (Z)-3-Hexen-1-ol, identified from the headspace of intact plants (Proffit et al., 2011) is found in very limited concentrations from normal tomato leaves. But this chemical compound was increased dramatically after damaging the leaves by different factors (Buttery et al., 1987). Similarly, several volatile compounds in tomato plants become much more abundant when *T. absoluta* lays its eggs on it. For instance, terpinolene was found only on insect-infested plants but not in normal plants (Angeles Lopez et al., 2012). Furthermore, a number of sesquiterpenes such as δ-elemene have been found to increase in the headspace of tomato plants with *T. absoluta* eggs (Anstasaki et al., 2015). However, these compounds are reported to play a role in tomato resistance against insects other than *T. absoluta* (Van der Hoeven et al., 2000; Bleeker et al., 2009). The production of such compound is aggravated when the environmental conditions are changed.

For instance, elevated emissions of sesquiterpenes are found after exposure of tomato plants under cold and hot stress (Copolovici et al., 2012). The discriminate analysis showed that these differences in ratios of similar blends can differentiate control against oviposited tomato plants, indicating an alteration of volatile blend after the oviposition with *T. absoluta* (Anstasaki et al., 2015).

The oviposition of *T. absoluta* shows significant preference and survival longevity towards tomato plants than other species of plants (Bawin et al., 2015). Tomato plants also give higher larval survivability and short development time as compared to the other plants (Bawin et al., 2015; Shehata et al., 2016). Additionally, the leaf contact also significantly increased the number of eggs laid, thus leaf surface morphology and chemistry accordingly have importance for oviposition of *T. absoluta* (Proffit et al., 2011). The leaf trichomes also provide chemical and mechanical stimuli for egg laying by female insect (Torres et al., 2001). The egg develops into first instar larva which starts to wound the leaf to enter into the mesophyll tissue of leaves or other parts of the host plant. The larval (feeding stages) development depends on the nutritional quality and/or production of plant metabolites (Bawin et al., 2015). The matured larvae pupate in the soil, on leaf and other parts of the plant. The pupa is initially soft, cylindrical, and green, later becomes dark brown (Genc, 2016). The pupal stage of the leafminer requires 8 to 15 days under controlled laboratory conditions. Adults become gray-brown and males are darker than females (Figure 1).

INSECTICIDE RESISTANCE MECHANISMS OF *T. ABSOLUTA*

Since *T. absoluta* was reported as leafminer of tomato in South America, insecticide spray was the primary controlling method. However, the insect becomes highly resistant towards synthetic insecticide and shows the reduced effectiveness of control (Lietti et al., 2005; Silva et al., 2011). This enforces farmers to apply different pesticides repeatedly on their farmland (Reyes et al., 2012). Such continuous and inappropriate chemical application on farmland aggravates the resistance problem on *T. absoluta* (Silva et al., 2011; Reyes et al., 2012). For instance, *T. absoluta* scored the higher resistant values 8-, 3.79-, 6.4- and 1.84-fold against four insecticides namely indoxacarb, metaflumizone, spinosad, and chlorantraniliprole in Turkey, respectively (Yalcin et al., 2015).

The chemical resistance of insect pest is mediated by enzymatic activities. Biochemical experiments suggest that the enzymatic systems might be involved in the loss of susceptibility of the tomato leafminer to different insecticides. For instance, insecticide spinosad that was registered in Chili has a unique mode of action against Lepidoptera larvae (Cleveland et al., 2002; Wang et al., 2009) and demonstrated low effectiveness towards *T. absoluta.* This is due to resistance enhanced by detoxifying enzymes such as mixed-function oxidases, glutathione-S-transferases, and esterases (Reyes et al*.,* 2012). Those enzymes are found in several insects and make them resistant to different insecticides (Rodriguez et al., 2010; El-Latif et al., 2010; Wu et al., 2011).

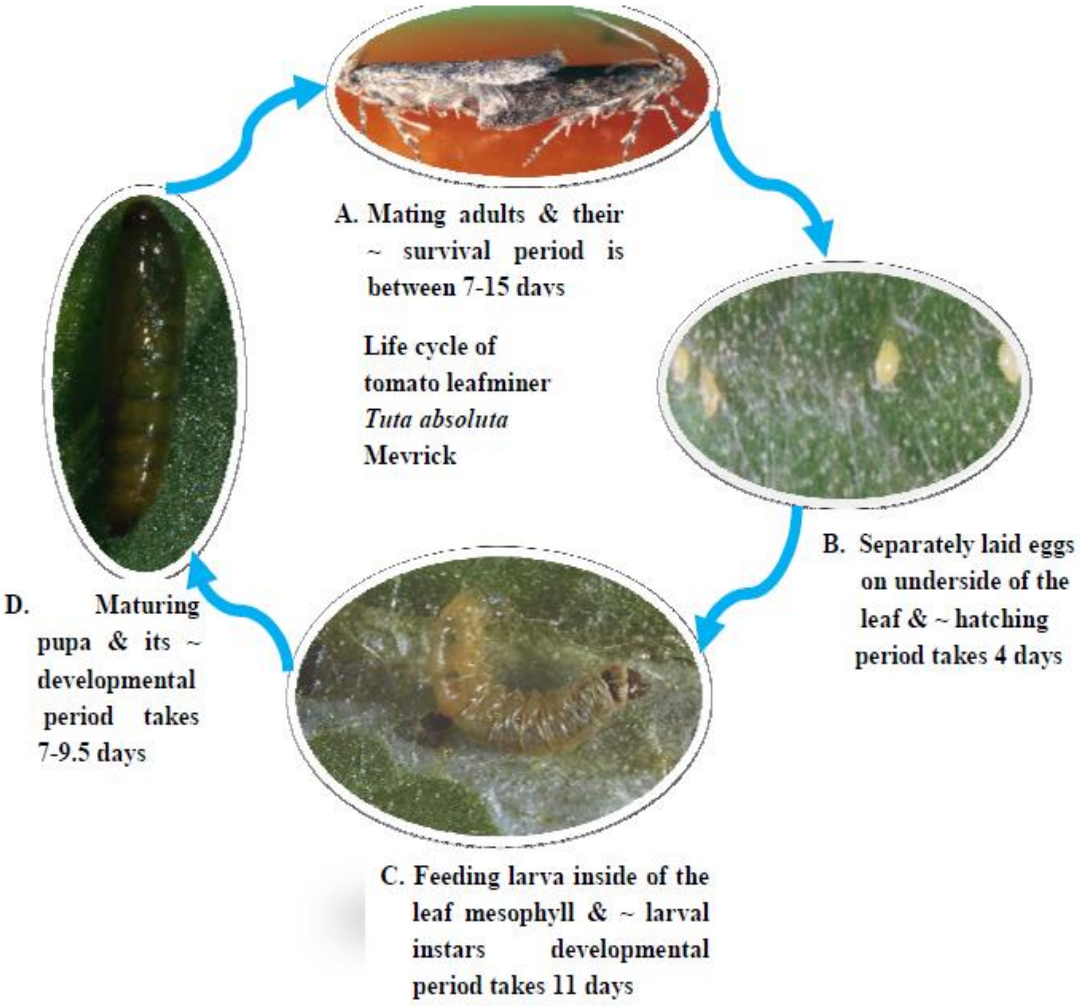

Figure 1. Life cycle of *T. absoluta*, photographs compiled from Arno et al. (2010) and developmental periods from Erdogan and Babaroglu (2014)

Esterases is non-specific enzyme implicated as an insecticide resistance mechanism in several insect pests due to their ability to hydrolyze insecticidal esters as organophosphates and pyrethroids (Soleno et al., 2008; Rodriguez et al., 2010; El-Latif and Subrahmanyam, 2010; Wu et al., 2011). The glutathione-S-transferase (GST) enzyme activity was determined to facilitate 1.5-fold higher resistance of *T. absoluta* against four insecticides, indoxacarb, metaflumizone, spinosad, and chlorantraniliprole. The esterase (EST) enzyme had a detoxifying activity of these four insecticides in equal performance (Yalcin et al., 2015). In another study, the toxicity of chlorpyrifos is synergized by diethyl maleate and triphenylphosphate. The synergistic effect ranged from 1.3 to 1.9-fold in 24 h and 1.2 to 1.5-fold in 48 h. The exposure with chlorpyrifos for 24 and 48 h significantly increased the activities of esterase. This suggested that esterase is involved in resistance through detoxification of chlorpyrifos in *T. absoluta*. It is confirmed in field populations of *T. absoluta* from Iran that displayed less susceptibility to chlorpyrifos (Zibaee et al., 2016). Furthermore, larval mortality in field was significantly lower by spinosad (47%) as compared to laboratory (91.7%) and the esterase effect ratio varied from 1.7 to 14.7 in the Chili (Reyes et al., 2011).

Moreover, diamide insecticides resistance in *T. absoluta* was reported and correlated to ryanodine receptors (molecular target-site) modification (mutation). It was investigated by reciprocally crossing resistant strain with a susceptible strain and revealed an autosomal incompletely recessive mode of inheritance. Genotyping of individuals of resistant and field-collected strains showing different levels of diamide resistance revealed that the presence of G4903E and I4746M RyR target-site mutations. The two novel amino acid mutations, G4903V and I4746T, in some of the resistant *T. absoluta* strains were detected. The functional performance evidence of these mutations is detected to alter the affinity of the RyR to diamides effect on *T. absoluta* (Roditakis et al., 2017).

IPM STRATEGY AND ITS EFFECTIVENESS

Environmental friendly controlling strategies have been developed for protection from *T. absoluta.* This includes, cultural control measures (crop rotation, selective removal and destruction of infested plant material) (Korycinska et al., 2009), using natural enemies (parasitoids, predators, entomopathogens and nematodes) (Desneux et al., 2010; Urbaneja et al., 2012), botanicals and production of resistant tomato cultivar varieties (De Oliveira et al., 2012). The integration of these methods with each other and if possible with less environmental hazardous insecticides are important to control *T. absoluta* without disturbing ecological world. Especially botanicals, natural enemies and microbial cells with their products are considered as promising alternatives than using synthetic pesticides.

The use of pheromone trap

Monitoring and mass trapping

Sex pheromone of insect is serving as alternative to monitor male moth population through trapping (Braham, 2014). Sex pheromone is chemical secretion released in the form of fluid that triggers the opposite sexual interest (Medido et al., 2013; Illakwahhi and Srivastava, 2017). This sex pheromone is released by virgin female tomato leafminer and strongly attractant to male moths (Quiroz, 1978). Mostly male moths are attracted to the secreted pheromone (Braham, 2014). Therefore, mass trapping of male moths is encouraged by using pheromone and imperative to early warning of pest abundance and monitor insect population (Megido et al., 2013; Ghoneim, 2014; Retta and Berhe, 2015). The trapped insects can be damaged mechanically or by applying chemical. These techniques have been successfully applied in controlling leafminers on both greenhouses and open field (Chermiti and Abbes, 2012; El-aassar et al., 2015).

Biochemical bio-pesticides (Plant extracts)

Botanicals are very important natural resources used to control different agricultural pests for long period of time. Crude extracts from seeds, leaves, bark, bulbs, and fruits of the different plant species have been extensively tested on agricultural pests for bioactivity worldwide (Isman and Seffrin, 2014). The phytochemical products, especially extracts or essential oils are suggested as potential alternatives than synthetic pesticides to control insect pests (Philogène and Vincent, 2005). Ethanolic leaf extract obtained from *Piper amalago* var. *medium,* for instance, caused 70% larval and pupal mortality in two-day exposure through exhibiting acute toxicity at the concentration of 2,000 mg L^{-1} (de Brito et al., 2015). The allelochemicals found in Piperaceae are diverse with insecticidal/insectistatic properties (Scott et al., 2008). From allelochemicals, piperamides are the major classes of compounds found in species of the genus *Piper* that exhibits dual biological activity on insects, neurotoxic and affect lipid metabolism (Dyer and Palmer, 2004) and promising to manage chemical resistant insect populations (Scott et al., 2004). Effects of the knockdown type, with paralysis and immediate death of the insect, has also been reported in insects treated with amides isolated from Piperaceae species (Scott et al., 2007).

The ethanolic extract from the Neem (*Azadirachta indica*) and petroleum ether extract from *Jatropha* (*Jatropha curcus*) seeds are tested on *T. absoluta* egg and larval stage as bio-insecticides (Kona et al., 2014). Thus, the ethanolic extract of Neem results in 24.5% egg and 86.7 to 100% larval mortality of *T. absoluta* at different concentration. The observed larval mortality is better than Neem Azal T/S 0.3% registered with active ingredient of azadiractin which kills 84 to 87% of first to fourth instars larvae after 14 days of exposure (Yankova et al., 2014). In the same way, the petroleum ether extract obtained from *Jatropha* also achieved 18 to 25% egg and 87 to 100% larval death on *T. absoluta* after being exposed for 4 days in different concentration (Moreno et al., 2011; Kona et al., 2014). The extracts obtained from jojoba (*Simmondsia chinsis*) seed at 100% concentration resulted in 75% mortality on 2nd larval instars of *T. absoluta* (Abdel-Baky and Al-Soqeer, 2017). The botanical extracts from “Tossegn” Garden thyme (*Thymus vulgaris*) and “Gulo” Castor bean (*Ricinus communis*) also caused 95 and 58% larval mortality of *T. absoluta,* respectively (Nilahyane et al., 2012). The aqueous extracts obtained from five different plant species showed moderate to high mortality on *T. absoluta* developmental stages. These extracts are from chinaberry (*Melia azedarach*) leaves and fruit caused (91%), geranium (*Pelargonium zonale*) (87%), garlic (*Allium sativum*) (85%), onion (*Allium cepa*) (80%), basil (*Ocimum basilicum*) flower (74%) and leaves (54%) mortalities on 2nd instars larvae at 6% concentration after 5 days exposure (Ghanim and Ghani, 2014). In another study, garlic also acts as a repellant for larvae in 37.5% and caused weight loss on first larval instars of *T. absoluta* (Salama and Shehata, 2017). This resulted in 78% 1st instars larval mortality after 4 days of exposure to 2000 ppm oil extract concentration. Clover (*Trifolium repens*) and *Eucalyptus* (*Eucalyptus camaldulensis*) oil extract also caused moderate (67 and 63%) mortality of 1st instars larvae at the highest (2000 ppm) concentration after 6 days exposure. Thus, crude extract concentration and exposure time are considered as the determinant factors for effectiveness of plant phytochemicals against *T. absoluta*.

The hexane and ethanol extracts from aerial parts of

Acmella oleracea exhibited the highest activity, causing (100 and 88%) mortality, respectively, in *T. absoluta* at a concentration of 10 µg of extract per mg of an insect after 6 h of exposure (Moreno et al., 2011). The bioactivity of *A. oleracea* is due to alkamides present in the plant. The main active amide in the plant is an isobutylamide (2*E*, 6*Z*, 8*E*)-*N*-isobutyldeca-2, 6, 8-trienamide, commonly known as spilanthol (Ley et al., 2006; Wu et al., 2008). Three alkamides were identified in the bioactive fractions of the hexane extract of *A. oleracea* [spilanthol, (*E*)-*N*-isobutylundeca-2-en-8, 10-diynamide and (*R*, *E*)-*N*-(2-methyl butyl) undeca-2-en-8,10-diynamide]. The results showed that all of the compounds isolated had a high insecticidal activity that was at least as toxic as permethrin, a pyrethroid recommended for control of *T. absoluta* (Moreno et al., 2011). The mechanism of action is that active alkamides found in *A. oleracea* has not yet been determined. However, it is roughly proposed that it affects the nervous system, as evident from abnormal movement like uncoordinated muscular activity. This effect suggests that the compounds disturb nerve conduction somewhere. Furthermore, crude extracts of three plant *A. indica* seed, *Cymbopogon citrates,* and *A. sativum* also caused 98, 97 and 95% mortality at larval stage of *T. absoluta*, respectively in 7 days of exposure in Ethiopia (Shiberu and Getu, 2017).

Biological control

The use of parasitoids and predators

The entomophagous insects, predators and parasitoids are natural enemies of insect and considered as alternative agents for pest control. As Luna et al. (2012) reviewed, more than 50 species of natural enemies are categorized under predators, parasitoids, and entomopathogens and used for *T. absoluta* control. Among these enemies, numerous studies are focused on parasitoid that approximately accounts for 20 species and considered as effective in Lepidopteran pest control (Polack et al., 2011). Moreover, six parasitoid groups are found to attack *T. absoluta* eggs, larvae and/or pupae (Luna et al., 2012). Specifically, Hymenopteran wasps, Tachinidae (Diptera) species, mirid bug *Tupiocoris cucurbitaceous* (Spinola) and the parasitoid wasps *Dineulophus phtorimaeae* (de Santis) and *Pseudapanteles dingus* showed positive traits on biological control aspect on insects (Luna et al., 2010; Lopez et al., 2011).

As investigators reported, *Trichogrammatoidea bactrae* and *Trichogramma pretiosum* are egg parasitoids of *T. absoluta* (Lewis et al., 2003). However, the endoparasitoid, *P. dignus,* and the ectoparasitoid, *D. phtorimaeae* formed more than 50% of natural parasitism in the larval stage of *T. absoluta* (Sánchez et al., 2009; Luna et al., 2010) and result in larval death due to nutrient consumption. The egg parasitoids are preferable since if they are effective, they can rapidly reduce the crop damage, whereas the larval and pupal parasitoids will control the pest once they result in the damage on the fruits, leafs and steams of the plant (Newton, 1998). Due to this, most of the reports are focused on egg parasitoids and they are dominantly categorized under Order Hymenoptera. The most important *T. absoluta* egg parasitoids are found in the families of Trichogrammatidae, Encyrtidae, and Eupelmidae (Ghoneim, 2014). About 210 species of *Trichogramma* are signaled as natural enemies of a variety of agricultural and forest pests in many regions of the world and at least 12 species are widely used commercially in biological control programs (Pratissoli et al., 2005; Kumar et al., 2009; Suckling and Brockerhoff, 2010). This parasitoid has short generation time and they can be easily reared on respective hosts (Mansour, 2010). *Trichogramma pretiosum* and *Trichogramma exiguum* are egg parasitoids under the family Trichogrammatidae and the most extensively used to control tomato leafminer *T. absoluta* (Desneux et al., 2010; Molla et al., 2011; Öztemiz, 2012; Vasconcelos, 2013). Furthermore, several reports indicated the existence of *T. absoluta* predators that participate in the population reduction. For instance, *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) regulates *T. absoluta* population due to its ability to prey efficiently on eggs (Molla et al., 2011). This predator species was able to prey on more than 100 eggs per individual per day (Arno et al., 2009). Two predator species, *Mesoglossus pygmaeus* and *N. tenuis* also reported as they reduced leaflet infestations up to 75 and 97% and fruit infestations up to 56 and 100%, respectively (Molla et al., 2009); though, preying on the egg and larvae of *T. absoluta* (Gadarra and Arno, 2010). However, until now there are no any reports that indicate the existence of adult parasitoids of *T. absoluta* (Ghoneim, 2014).

The susceptibility of insect larvae and pupae to entomopathogenic nematodes (*Steinernema carpocapsae*, *Steinernema feltiae*, and *Heterorhabditis bacteriophora*) was reported (Grewal et al., 2005). This is indicated in leaf bioassays evaluation of tomato using the nematodes for the capability to reach the larvae and kill them within the galleries (Rodriguez et al., 2005). Such experimental evaluation showed high larval mortality (79 to 100%) and low pupae mortality of only 10% (Rodriguez et al., 2005; Batalla-Carrera et al., 2010). In the leaf bioassay, a high level of larval parasitization (77 to 92%) was recorded revealing the nematode’s capacity to kill the larvae inside the galleries. The pot experiments indicated that nematode treatment reduced insect infection of tomato plants by 87 to 95% (Batalla-Carrera et al., 2010).

The use of microbial cell

The plant growth promoting the effect of soil microbes has been shown to affect plant-insect interactions (Pineda et al., 2010). Improvement of the nutrient composition increases plant nutritional quality, while it affects insect performance at several trophic levels (Schoonhven et al., 2005; Zhang et al., 2008). Systemic responses that plant root microorganisms cause emission of volatiles at aerial parts of plants in multitrophic interactions that attract above-ground parasitoids (Bruce and Pickett, 2007). Furthermore, plants interacting with beneficial microbes can also benefit from an increase in tolerance to herbivory (Bennett et al., 2008; Vannette and Hunter, 2008). This can be achieved by improving nutrient and water uptake facility for better plant development. Furthermore, beneficial microbes can facilitate the regrowth of tissues after herbivory and promote plant tolerance, which is reflected in compensation of the loss of plant biomass in the presence of herbivores (Herman et al., 2008; Kempel et al., 2009). For instance, *Trichoderma* species is plant growth promoting fungus that is commercially available and widely used in agriculture that induces systemic resistance against microbial pathogens in plants (Harman et al., 2004; Trillas et al., 2009; Segarra et al., 2009). When the *Trichoderma* spp. interacts with the plants, they recognize the existence of the species through the signal communication and become ready to defend other invaders. However, the plant-mediated effects on insects are not assessed (Pineda et al., 2010).

There are several soil microbes that have the ability to induce plant resistance in systemic tissues (induced systemic resistance, ISR). Induced systemic resistance in the plant is resulted by plant growth promoting bacteria, *Pseudomonas* and *Bacillus* species (Van Wees et al., 2008; Van der Ent et al., 2009), root mycorrhizal fungi (Trillas et al., 2009), areal stem endophytic fungi (Stein et al., 2008) and soil-plant growth promoting fungi (Segarra et al., 2009). The ISR efficiency is proven in different plant species against pathogenic bacteria, fungi, viruses, nematodes and recently insect pests (Van Oosten et al., 2008; Stein et al., 2008). The priming defense genes show a higher expression systemically in the leaves only after pathogen or insects attack in responsiveness of the plant to the plant hormones jasmonic acid and ethylene (Van Wees et al., 2008; Van der Ent et al., 2009). The levels of these hormones remain unchanged, suggesting that ISR is based on increased sensitivity to plant hormones rather than on the increased production of such hormones (Van der Ent et al., 2009).

Priming is a phenomenon that provides plants with an enhanced capacity to rapidly and effectively mount defense responses to biotic and abiotic stresses (Conrath et al., 2006). It is elicited by beneficial microbes, but also by pathogens, herbivores and selected synthetic compounds such as benzothiadiazole and b-aminobutyric acid (Heil and Silva, 2007; Frost et al., 2008). Typically, priming is characterized by accelerated defense-related gene expression once primed plants are attacked by a pathogen or an insect, resulting in an enhanced level of resistance against the invader (Pineda et al., 2010). This enhanced resistance is effective against a broad spectrum of attackers. The molecular basis of priming is poorly understood, but recent advances in the field of priming demonstrate that the accumulation of latent defense-related transcription factors (Van der Ent et al., 2009), mitogen-activated protein kinases (MAP kinases) (Beckers et al., 2009) and secondary metabolites such as azelaic acid (Jung et al., 2009) and volatile organic compounds (Heil and Silva, 2007) participate on establishment of the primed state.

The use of microbial product

The bacterium *Bacillus thuringiensis* is a gram-positive spore-forming bacterium that produces toxic crystal proteins (Glare, 2000). This protein is a delta-endotoxin protein that is eaten by insect larvae. Then the protoxin is dissolved in the parasporal bodies and activated under alkaline conditions in the mid-gut of target insects, thereby releasing the active peptides that bind to specific receptors in the insect’s mid-gut epithelial cells and creating pores in the epithelial membrane and stop insect feeding and ultimately result in death due to starvation (Charles et al., 1996). Thus, this bacterium produces such protein crystals during sporulation and the product is toxic to insect larvae and causes rapid death (Rezaei and Talaei-Hassanloui, 2016). The pure protein crystals from five *B. thuringiensis* strains that were taken from dead or diseased larvae exhibited satisfactory efficacy against *T. absoluta* larvae (Medeiros et al., 2006).

B. thuringiensis that was isolated from local soil show three folds toxicity against *T. absoluta* larvae compared to one of commercial *B. thuringiensis* in Algeria (Hernandez-Fernandez et al., 2010). Furthermore, four (B1, B2, B3, B4) *Bacillus* spp. isolated from dead larvae in Egypt caused the larval mortality of *T. absoluta* at 4th instars and the effect ranged between 80 and 93% in seven days interval (Youssef and Hassan, 2013). The effectiveness was increased (96.7%) at a concentration (2 g/ml) of isolates (Youssef and Hassan, 2013). This is an indicator of crystal protein that as it increase the concentration of *Bacillus* spp., it increases the mortality. Toxins expressed by *Bacillus* spp. naturally colonize the phylloplane of tomato plants and survive for a period of 45 days on the tomato leaf surface (Theoduloz et al., 2003) that result in mortality on first to fourth larval instars, however, the death variation occurred due to feeding behavior difference (Giustolin et al., 2001; Youssef and Hassan, 2013). The first and second larval instars penetrate directly the leaves without much feeding. Therefore, they are exposed to a lower dose of bacterial spores and toxins. Older instars are more susceptible to treatments than younger ones due to their longer stay or larval movement in and out of the mines and galleries several times during their development and at that moment they are very vulnerable to infection by the bacterial spores (Harizanova et al., 2009).

In the field condition, *B. thuringiensis* after 50 days of the application showed a significant average reduction of *T. absoluta* infestation to 12.4%, individuals as compared to 26.5±11.34 individuals in the control. Furthermore, after 90 days of the post applications, the means number of infestations significantly decreased to 46% as compared to 30.6 individuals in the control. The weight of the tomatoes was determined as better scored after harvest (Sabbour and Soliman, 2012).

The entomopathogenic fungus *Baeuveria bassiana* was considered as the best and effective candidate against larvae of *T. absoluta*. These fungal strain, with high dose treatment (4.75×10^7 conidia/ml), resulted in 100% larval death within 3 days, while low doses led in a fatality rate of 87% within 4 days (Ikram and Abdallah, 2011). Another investigator also reported that *B. bassiana* that caused 68% *T. absoluta* death at larval stage (Cabello et al., 2009) *Metarhizium anisopliae* also kills 37% of female moths (*T. absoluta*) (Cristina et al., 2008). In Egypt, the used *M. anisopliae* and *B. bassiana* reduced *T. aboluta* infestation by 9.8± 9.9 and 10.8±11.9 individuals as compared to 21.7 ±8.6 individuals in the control under greenhouse conditions (Sabbour, 2014).

RNA INTERFERENCE (RNAI)

The RNA interference (RNAi) is a mechanism that small RNAs can rapidly cause post-transcriptional specific gene silencing (Hannon, 2002). Such biotechnological approach is currently becoming the potential application for agricultural insect pest control (Gordon and Waterhouse, 2007; Katoch et al., 2013). Tomato pinworm, *T. absoluta* mitigation was initiated by targeting on gene silencing approaches using small RNA interference (Price and Gatehouse, 2008; Camargo et al., 2016). RNA interference (RNAi) gene silencing process is performed by providing double-stranded RNA (dsRNA) molecules that match a specific target gene sequences in a targeted organism (Koch and Kogel, 2014).

This approach works when the plant was engineered to express insect dsRNAs (Price and Gatehouse, 2008). However, obtaining sufficient, effective and essential genes used for insect development and survival was a critical step (Zhang et al., 2013). Thus, the first gene screening from *T. absoluta* was made using insect hormone biosynthetic pathways as potential targets for systemic silencing (Camargo et al., 2015). The production of transgenic plants expressing dsRNA molecules targeted to essential insect genes was providing a means of specific gene silencing in plant-feeding larvae. Then the larval phenotypes are developed loss of appetite to death (Camargo et al., 2016). The first experiment of such method on the *T. absoluta* was conducted by Camargo et al. (2016) using two approaches to dsRNA delivery for larvae. The supply technique is allowing larvae to feed upon the tomato leaves that contains dsRNA obtained through absorption from aqueous solution through sap-sucking of *Bemisia tabaci* (Luan et al., 2013) and transient transcription of dsRNA by the host plant after the infiltration of *Agrobacterium* cells carrying binary plasmids that expressed hairpin versions of the target gene sequences (`agro-infiltration') (Leckie and Stewart, 2010). This method is designated as "*in planta-induced transient gene silencing"* (PITGS) that successfully delivered hairpin silencing genes in other pathogens (Panwar et al., 2012).

The Vacuolar ATP*ase* catalytic subunit A gene (V-ATPase) and Arginine kinase were targeted gene for this experiment (Camargo et al., 2016). The H^+-ATPase vacuolar pump is one of the essential enzymes present in almost all eukaryotic cells that are responsible for generating energy gradients in many membranes and organelles (Nelson et al., 2000; Wieczorek et al., 2009). Moreover, arginine kinase belongs to a transferase protein family that catalyzes the transfer of a high energy phosphate group from ATP to L-arginine to yield phosphor-arginine used for energy storage (Bragg et al., 2012; Kola et al., 2015). Then the report was indicating that silencing of the insect V-ATPase and Arginine kinase genes by dsRNA showed 60% reduction in target gene transcript accumulation and increase larval mortality (Camargo et al., 2016). Development of transgenic tomato lines expressing the hairpin version of these genes demonstrated that this approach can adversely affect insect development and viability by reducing the expression of insect target genes. Thus, leaf-absorbed dsRNA delivery avoids cloning steps into specific vectors and allows a better control of provided dsRNA amounts in the leaves (Luan et al., 2013; Camargo et al., 2016), whereas PITGS offers a more realistic trigger of the RNAi machinery in transgenic plants, since the dsRNA hairpin molecule will be transcribed and processed by the plant cells (Camargo et al., 2016).

The findings are suggesting that in addition to the easy provision of dsRNAs for insect pests, sufficient dsRNA accumulation is also required to produce gene silencing in *T. absoluta* at least minimum dose before triggering RNAi (Yu et al., 2012). This requires trans-membrane proteins, such as systemic RNA interference deficient-1 proteins (SID-1) that involved in dsRNA uptake and systemic spreading in the tissues (May and Plasterk, 2005) and then putative orthologous SID-1 genes in the *T. absoluta* transcriptome was identified by RNA-seq analysis (Camargo et al., 2015) for transportation.

INHERITED STERILITY TECHNIQUES (IST)

Inherited sterility techniques are commonly practiced to control different agricultural insect pests (Bloem and

Carpenter, 2001; Carpenter et al., 2005; Boshra, 2007). It does not exert any effect on the environment and impotent approach to control especially invasive pest in an agricultural system like *T. absoluta* (Cagnotti et al., 2012). The radiation sterilization of insects and sterile male insect release into a wild population of the same species was expected to prevent the reproduction of wild females and practiced for pest control (Knipling, 1955). At the first time such sterilization has used ɤ-radiation with a highly radioactive material, although, it is currently not available in the market due to difficulties related to the transportation of co-sources (Mastrangelo et al., 2010; Mehta and Parker, 2011). Alternatively, X-irradiators are used for sterilization of insect pests. It is easier and independent of a radioactive source that is performed safely on insect sterility (Cagnotti et al., 2012).

The inheritance sterilization works in the way that the irradiated male insect is released into the natural population and mate with an untreated female to produce abnormality in offspring. In this case, most radiation-induced chromosomal breaks will not lead to the loss of chromosome fragments and remains stable during both mitosis and meiosis and can be transmitted through germ cells to the next generation (Marec et al., 2001; Carpenter et al., 2005). When partially sterile males mate with wild fertile females their deleterious effects induced by radiation are inherited and expressed for several generations, mainly in the first generation of descendants of irradiated adults (Carpenter et al., 2005). Such practice is firstly conducted by Cagnotti et al. (2012) on the *T. absoluta* and considered as an effective technique to control it. The optimum dose of X-radiation to make male moth sterile is between 200 and 250 Gy (Cagnotti et al., 2012). In the first generation, the egg hatching is reduced and the produced offspring becomes highly sterile (more than the irradiated parent moths) and most are male (Bloem and Carpenter, 2001; Seth and Sharma, 2001). Moreover, adult emergence decreased at doses of X-radiation increased the results into formation of malformed wings and bent legs at doses >350 Gy (Cagnotti et al., 2012).

Thus, the inherited sterility of insect pest technique is considered as a key strategy to manage *T. absoluta* and it is considered as the area-wide concept of integrated pest management system (Carpenter et al., 2005; Cagnotti et al., 2012). This inherited sterility way of pest management is compatible with the use of other area-wide control tactics such as mating disruption, biological control, cultural control methods, and the use of bio-rational pesticides (Bloem and Carpenter, 2001; Carpenter et al., 2005).

SYNERGETIC RESISTANCE DEVELOPMENT OF HOST TOMATO

Insect resistant cultivar development is considered as one of alternative approach than chemical pesticides for obtaining cost-benefit ratio for pest control (Pereira et al., 2008; Gonçalves et al., 2010; Maluf et al., 2010). In Brazil, tomato improvement was practiced to obtain pest-resistant cultivars by incorporating the alleles for resistance present in wild plants for commercialization and produce the allele-chemicals associated with resistance (Silva et al., 2009; Gonçalves et al., 2010; Maluf et al., 2010). Three types of allele-chemicals (acyl-sugars, zingiberene, and 2-tridecanone) have been associated as resistant to *T. absoluta* (Pereira et al., 2008; Silva et al., 2009; Maluf et al., 2010). However, studies comparing levels of pest resistance among strains rich in different allele-chemicals are few (De Oliveira et al., 2012).

The resistance of tomato strains rich in 2-tridecanone (2-TD), zingiberene (ZGB), and acyl sugars (AA) to the tomato moth, *T. absoluta* showed significant reduction on evaluated characteristics, such as oviposition rate, severity of damage to the plants, injuries to the leaflets, and the percentage of leaflets attacked (De Oliveira et al., 2012). The oviposition rate reduction of *T. absoluta* was directly associated with high concentrations of 2-TD, ZGB, and AA in the tomato cultivars (Silva et al., 2009; Gonçalves et al., 2010; Maluf et al., 2010). This is a very important strategy to prevent the tomato plant from insect attack. These three allele-chemicals had equivalent effects on oviposition, which was consistently lower on these strains than on the susceptible controls (De Oliveira et al., 2012).

CONCLUSION

The agricultural pests are main threats for the vegetables, fruits, cereals and flower production in several countries. *T. absoluta* is one of the invasive tomato pest and it is devastating worldwide. The insect will result in 80 to 100% crop damage if it is not controlled. The entrances of larvae into leaf mesophyll prevent them from pesticides and save them from death. Furthermore, *T. absoluta* has developed resistance to different types of pesticides and become too difficult to control it using insecticides. The repeated spraying of synthetic pesticides on the farmland is becoming problematic to environmental safety.

The IPM strategy is very important and has dual purpose, environmental friendly and effective to manage insect pests. Biological control is one of IPM and potential to mitigate insect pests from farming system. Microbial groups both bacterial and fungal species are showing important implication on *T. absoluta* control. Furthermore, biotechnological techniques are also another alternative to control tomato leafminer *T. absoluta*. It is a newly coming technology mostly targeted on RNA interference. Small RNA interference that hairpin formation of introduced double serenaded RNA interfaces the essential component synthesis in the insect and stop the insect growth. Thus, IPM strategy is then the promising alternative to control *T. absoluta* for healthy farming of the tomato vegetable.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Full Length Research Paper

The effect of inter cross-inoculation host group rhizobia on the growth and nitrogen fixation of Faba Bean (*Vicia faba L.*) varieties in North Showa, Amhara Regional State, Ethiopia

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Faba bean (*Vicia faba* L.) is one of the most widely cultivated leguminous crops grown in the Highlands of Ethiopian. This study was aimed at evaluating the effect of *Rhizobium leguminosarum bv.viciae* isolates from other cross-inoculation hosts from field pea, grass pea, and lentil on faba bean varieties. Thus, 12 isolates were selected from faba bean, field pea, grass pea and lentil using host trap method on soil samples that was collected from North Shoa. All isolates were tolerated to pH (5 to 9), salt concentration (1 to 2%), and at a temperature of (15 to 35°C). Cross-inoculation experiments were conducted in the greenhouse to test the effects of *R. leguminosarum* isolates from faba bean, field pea, grass pea and lentil hosts on Hachallu and Wolki varieties of faba bean. The mean nodule number, nodule dry weight and mean shoot dry weight of the inoculated plants showed variations among the different cross inoculants ($p<0.01$). Symbiotic effectiveness varied from a minimum of 64% on Hachallu variety inoculated by isolate AUFBR5 (faba bean) and 52% on Wolki variety by isolate AUFPR1 (field pea) association to a maximum of 98% in Hachallu variety inoculated with isolate AUGPR13 (grass pea), and 117% in Wolki variety inoculated with isolate AUFBR5 (faba bean). The study showed physiological and symbiotic diversity of *R. leguminosarum* isolates of faba bean, field pea, grass pea and lentil. The effective isolates that have been tested with Hachallu and Wolki varities at green house level should be re-tested under field conditions to ascertain their performance *in vivo*.

Key words: Field pea, grass pea, lentil, symbiotic effectiveness.

INTRODUCTION

Legumes are one of the most diverse plants on earth which widespread in tropics and temperate zones (Tran and Nguyen, 2009). Leguminosae family comprises 800 genera and 20,000 species, and represents the third

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largest family of flowering plants (Siddique et al., 2012). Faba bean (*Vicia faba*) is one of the major cold season food crops grown in Ethiopian Highlands (CSA, 2005). Faba bean has different functions such as providing food and feed that is rich in protein improve soil fertility by symbiotic N_2 fixation with Rhizobium, diversifying the cropping system with crop rotation by reducing constraints on growth and yield of other crops (Nikfarjam and Aminpanah, 2015).

Ethiopia is the world's second-largest faba bean producer next to China (Salunkhe and Kadam, 1989) with annual production of 2,671,834.45 ton from 1,558,422.02 ha of land (CSA, 2015). However, the productivity of faba bean in Ethiopia is still, far below its potential due to biotic and biotic factors (Yohannes, 2000). Amongst the various limiting factors affecting plant growth and productivity, nitrogen deficiency in soil is one of the most common factors for a reduction in yield of legume crops such as faba bean particularly in Ethiopia (Adler, 2008). The supply of nitrogen into the soil must be sufficient to increase the yield of plants (Ihsanullah et al., 2008). Chemical nitrogen fertilizers are very expensive and had an adverse effect on the environment and human health. Hence, there is an urgent need to realize a vital and cheaper source of fertilizers having an eco-friendly approach. Biological nitrogen fixation with rhizobia-legumes symbiosis is one of the alternative solutions and the promising technologies (People, 1995).

Faba bean is the most effective nitrogen fixer of all cool-season pulse crops grown in association with root nodule bacteria (*Rhizobium leguminosarum* bv*.viciae*) (McVicar et al., 2005). Faba bean is nodulated by *Rhizobium leguminosarum bv. Viciae* that derives the highest percentage (96% Ndfa) of nitrogen from the atmosphere (Lo´ pez-Bellido et al., 2006). Jensen et al. (2010) reported that the crop comes after faba bean benefits up to 100 to 200 kg N ha^{-1}. It has been reported that the rhizobium inoculation increases the production and protein content of faba bean (Giller, 2001).

The specific rhizobium that nodulates faba bean is *Rhizobium leguminosarum* bv*.viciae* which also cross-nodulates field pea, grass pea, and lentils (McVicar et al., 2005). The finding showed that Rhizobia strain that is effective in nitrogen fixation in association with one plant species may also be effective with certain other legumes (Amara, 1990). This is demonstrated by isolates of pea and faba bean able to form nodules and fix nitrogen with faba bean host (Laguerre et al., 2003). However, due to the influence of host plant on diversity and the genetic structure, *R. lguminosarum bv.viciae* reflect differences in the degree of host specificity and effectiveness of nitrogen fixation within the *viceae* tribe cross-inoculation group (Handley et al., 1998).

In Ethiopia, research was conducted on cool season legumes such as faba bean, lentil, and field pea (Hailemariam and Tsigie, 2006). However, most of the studies were limited to isolation, identification, and characterization of rhizobial isolates from faba bean in different parts of the country (Belay and Assefa, 2011). As a result, there is lack of sufficient information about cross-inoculation of rhizobia isolated from nodules of viceae tribe cross-inoculation group for their effectiveness in faba bean varieties. Hence, this study was directed to isolate and evaluate the effectiveness of inter cross-inoculation host group rhizobia on the growth and nitrogen fixation of faba bean (*Vicia faba* L.) varieties under greenhouse condition.

MATERIALS AND METHODS

Sample collection

Soil samples were collected from twenty sites in North Shoa Zone of Amhara Region, Ethiopia. From each site, two farmer fields were selected and samples from 10 to 15 cm depth were pooled and collected in alcohol sterilized polyethylene plastic bags. Types of legumes on the soil field were Faba bean, Field pea, Grass pea and lentil (Table1). The soil samples were brought to Applied Microbiology Laboratory of Addis Ababa University for further processing. The Seeds of Wolki and Hachallu varieties of faba bean (*Vicia faba*), Adi variety of field pea (*Pisum sativum*), and Alemaya variety of lentil (*Lens culinaris)* were provided by Holeta Agricultural Research Center whereas Wasse variety of grass pea *(Lathyrus sativus*) was gotten from Debrezeit Agricultural Research Center (Table 1).

Isolation of *Rhizobium* from nodules

The soil samples were filled into 3 kg capacity plastic pots. The seeds of faba bean (*Vicia faba*), field pea (*Pisum sativum*), lentil (*Lens culinaris***)** and grass pea *(Lathyrus sativus*) were surface sterilized and sown in each pot under greenhouse conditions. After 45 days, the pink and undamaged nodules were collected and surface sterilized (Lupwayi and Haque, 1994). Nodules were crushed with alcohol flamed sterile glass rod (Somasegaren and Hoben (1994). A loopful of crushed nodule saps were streaked on Yeast Extract Mannitol Agar (YEMA) plates and incubated at 28±2°C for 3 to 5 day. According to Somasegaren and Hoben (1994), Yeast Extract Mannitol Agar (YEMA) medium of volume 1000 ml consists of the following components (g/l):

Mannitol, 10; K_2HP0_4, 0.5;$MgS0_4 .7H_20$, 0.2;NaCl, 0.1;Yeast Extract, 0.5; Agar,15.

The purity and uniformity of colony types were carefully examined through repeated re-streaking. Pure culture was preserved on YEMA slant containing 0.3% (W/V) $CaCO_3$ at 4°C for future use (Jordan, 1984).

Presumptive tests and designation of the isolates

The growth of isolates was evaluated on the peptone-glucose agar medium (Lupwayi and Haque, 1994). The purity of isolates was tested on Congo -Red (CR-YEMA) (Somasegaren and Hoben, 1994). All the isolates were gram stained, and their shapes and gram reaction were observed microscopically for rapid means of identification of contaminants as indicated in Lupwayi and Haque (1994). All the isolates were designated at Addis Ababa University Grass pea Rhizobia (AUGPR), Addis Ababa University Field pea

Table 1. Sites of soil sample collection and the types of legumes on the soil field.

S/N	Sample sites	Collection zones	Types of legumes on the soil field
1	Cheki	North Shoa	Faba bean
2	Bedeyo	»	Faba bean
3	Jajo	»	Faba bean
4	Kulso	»	Faba bean
5	Dalota	»	Faba bean
6	Kulso	»	Field pea
7	Dalota	»	Field pea
8	Cheki	»	Field pea
9	Bedeyo	»	Field pea
10	Jajo	»	Field pea
11	Segeda	»	lentil
12	Amino	»	lentil
13	Adadi	»	lentil
14	Hawi	»	lentil
15	Folle	»	lentil
16	Adadi	»	Grass pea
17	Hawi	»	Grass pea
18	Folle	»	Grass pea
19	Amino	»	Grass pea
20	Segeda	»	Grass pea

Rhizobia (AUFPR), Addis Ababa University Faba bean Rhizobia (AUFBR), and Addis Ababa University Lentil Rhizobia (AULR) representing each isolate.

Authentication of isolates on sand culture

In order to test the definitive purity of all rhizobial isolates, nodulation test was carried out for each of the purified isolates. They were inoculated into the host plant potted into 3 kg capacity plastic pots containing sterilized and nitrogen free sand (Somasegaren and Hoben, 1994). The sand was thoroughly washed with sulfuric acid whereas the pots were surface sterilized with 95% ethanol. Six seeds were sown in each pot and thinned down to three after germination. Each isolate was inoculated into Erlenmeyer flask for 3 days, and 1 ml of the culture suspension was inoculated into each seedling. The control plants were fertilized with 100 ml of 0.05% KNO_2 once every week. All plants were irrigated with N-free medium nutrients four times in their life (Broughton and Dilworth, 1971).

Morphological and cultural characterization of isolates

Colony morphology was characterized on YEMA as indicated in Martinez-Romero et al. (1991). The ability of isolates to produce acid/alkaline evaluated in the YEMA medium containing bromothymol blue (BTB) (0.025w/v) (Jordan, 1984). The growth rate of the isolates was determined as described by White (1995).

Physiological characteristics of isolates

Tolerance to acidity and alkalinity of each isolate was evaluated on YEMA media adjusted to pH levels of 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11 and 12 **(**Bernal and Graham, 2001). The ability of the rhizobial isolates tolerance to NaCl was tested by streaking on YEMA plates containing 1, 2, 3,4, 5, 6, 7, 8, 9 and 10% NaCl (w/v) as indicated in Bernal and Graham (2001). The growths of isolates were evaluated on YEMA plates at a different incubation temperature of 15°C, 20°C, 25°C, 30°C, 35°C, 40°C and 45°C (Lupwayi and Haque, 1994).

Cross-inoculation and symbiotic effectiveness test on sand culture

Rhizobial isolates of faba bean, field pea, grass pea, and lentil were selected as representative on the basis of the agronomic and economic importance of their legume host in the country (Table 2). They tested for their ability to form nodules and effective symbiosis with Hachalu and Wolki varieties of faba bean (*Vicia faba*). The sand was washed with sulfuric acid and autoclaved for sterilization whereas pots were surface sterilized with 95% ethanol (Lupwayi and Haque, 1994). The seeds of Hachalu and Wolki varieties of *Vicia faba* were rinsed in 95% ethanol for 10 s, and surface sterilized in 3% (v/v) solution of sodium hypochlorite for 3 min (Lupwayi and Haque, 1994).

Seeds were washed with sterile distilled water and planted into each pot. Starter cultures of selected test isolates were grown in test tubes containing 10 ml YEM broth on an orbital shaker for 3 days. 1 ml YEM broth culture of each test isolates was transferred into 100 ml sterilized YEM broth in 250 ml Erlenmeyer flask, and placed on an orbital shaker for 4 days. 1 ml of each 4 days of YEM broth culture was inoculated on to the base of seedlings of *Vicia faba* according to Vincent (1970).

The treatments were arranged in a randomized complete block design in triplicate. After 45 days of growth, the whole plants were carefully uprooted to determine modulation number, nodule dry weight, and shoot dry mass according to Vincent (1970) (Table 2). The shoots dry weight was determined as described by Somasegaren and Hoben (1994). The effectiveness of the isolates

Table 2. Selected isolates from viceae tribe host legumes for cross-inoculation test.

S/N	Isolates	Site	Host of isolates
1	AUFBR2	Bedyo	Faba bean
2	AUFBR3	Jajo	Faba bean
3	AUFBR5	Daloda	Faba bean
4	AUFPR6	Kulso	Field pea
5	AUFPR7	Dalota	Field pea
6	AUFPR10	Jajo	Field pea
7	AUGPR12	Amino	Grass pea
8	AUGPR13	Adadi	Grass pea
9	AUGPR14	Segeda	Grass pea
10	AULR16	Adadi	Lentil
11	AULR17	Hawi	Lentil
12	AULR18	Fole	Lentil

in accumulating plant shoot dry matter was calculated as described in Mulongoy (2004).

$$\%SE= \frac{\text{Inoculated plant DM}}{\text{N-fertilized plant DM}} \times 100$$

Where, DM = dry matter, N= nitrogen, SE= symbiotic effectiveness The rate of nitrogen-fixing effectiveness is evaluated as highly effective > 80%, Effective from 50 to 80%, lowly effective 35 to 49% and infective <35%.

Data analysis

Symbiotic effectiveness parameters such as nodule number, nodule dry weight, and shoot dry weight data were analyzed by one-way ANOVA (Tukey's HSD test) SPSS (Somasogaran and Hoben, 1994).

RESULTS AND DISCUSSION

Presumptive test of isolates

In this particular study, 20 isolates from faba bean, field pea, grass pea and lentil using host trap method on soil samples were collected from North Shoa. They were authenticated and characterized on the basis of their different phenotypic and symbiotic characteristics. None of the isolates grew on peptone-glucose-agar (PGA). Similarly, Somasegaren and Hoben (1994) stated that PGA does not allow the growth of rhizobia. All isolates were gram-negative and rod-shaped bacteria with no absorption of Congo-red from the YEM-CR medium (Jordan, 1984).

Authentication and relative effectiveness of rhizobia isolates on sand culture

On the basis of the nodule formation and symbiotic effectiveness of isolates in their respective host, nodule number, nodule dry weight and shoot dry weight were determined. All of the isolates except AUFBR4 (faba bean) and AUGPR11 (grass pea) were authenticated as root nodule bacteria from sand culture on the host plants according to Vincent (1970) and Brockwell (1998) (Table 3). The failure to re- nodulation could emanate from loss of symbiotic plasmids (Sym-plasmids) that govern the symbiotic interaction between the two partners (Zhange et al., 2001). As a result, those isolate that failed to re-modulate with their parent host may be due to other intruding bacteria that penetrated The shoots dry weight was determined as described by Somasegaren and Hoben (1994). The effectiveness of the isolates

Morphological and cultural characterization of isolates

Colony morphology, colony diameter, acid-base reaction and generation time were used to evaluate the diversity of the test isolates. The different isolates showed variations amongst one another in their characteristics (Table 4). Isolates were fast growers with large mucoid and watery texture, and colony diameter of 2.5 to 6mm, the generation time of 1.0 to 4.3 h. They changed YEMA-BTB into yellow. Several works showed that *R. leguminosarum* bv.*viciae* nodulating faba bean (Adamu et al., 2001; Shimekit et al., 2008) were identified as fast-growing root nodule bacteria. Since they were isolated from faba bean, field pea, grass pea and lentil, they were also characterized as a cross nodulating group of *R. leguminosarum* bv. *viciae* (Jordan, 1984).

Physiological characteristics of rhizobial isolates

The inhibitory effect of salt concentrations varied among isolates of faba bean, field pea, grass pea and lentil (Table 5). The result indicated that the percentage of tolerance continued to decrease as the concentrations of NaCl increases. All 100% of the isolates grew at 1 to 2% salt concentrations. Some isolates which included AUFBR2 (faba bean), AUGPR13 and AUGPR14 (grass pea) were found to grow at all tested salt (1 to 10%) levels of concentrations as described in Adal (2009). Adal (2009) work on grass pea rhizobia reported that some isolates of *R. leguminosarum* can survive in the presence of extremely high levels of salt concentrations up to 13% of NaCl. In contrast, Teshome (2006) reported that faba bean isolated strains of *R. leguminosarum bv. Vicieae* managed to grow at up t o 6% NaCl from the Northern Ethiopia.

Isolates were grown at a different range of pH (pH 4-12) (Table 5). All isolates were tolerant to pH 5 to 9 according to Demissie (2006). Demissie (2006) reported that the *R. leguminosarum biovar Vicieae* of *Vicia faba* and *Pisum sativum* from Northwestern Ethiopia grew at pH more than 4.75. Isolates AUFBR5 (faba bean),

Table 3. Authentication and relative effectiveness of rhizoidal isolates.

Isolates	Site	Host	Nodule number/ plant	Nodule dry weig ht/plant (mg)	Shoot dry weight/plant (g)	Percentage (%) SE	Effectiveness
AUFBR1	Cheki	Faba bean	25±2.88b	6±0.001^{a}	2.8±0.12^{a}	76	E
AUFBR2	Bedeyo	″	70±5.77^{c}	89±0.006^{b}	3.01±0.11^{bc}	83	HE
AUFBR3	Jajo	″	118±6.11^{e}	90±0.004^{b}	3.17±0.05^{cd}	87	HE
AUFBR4	Kulso	″	-	-	1.50±0.10^{a}	41	LE
AUFBR5	Dalota	″	90±5.77^{d}	88±0.006^{b}	3.52±0.18^{d}	96	HE
(+)Control		″	-	-	3.65±0.09^{de}		
AUFPR6	Kulso	Field pea	105±5.51^{d}	37±0.009^{c}	1.13±0.40^{a}	73	E
AUFPR7	Dalota	″	120±2.65^{d}	54±0.006^{d}	1.56±0.28^{a}	101	HE
AUFPR8	Cheki	″	25±5.13^{b}	10±0.001^{ab}	1.01±0.31^{a}	65	E
AUFPR9	Bedeyo	″	20±5.29^{b}	7±0.001^{ab}	0.54±0.37^{a}	35	LE
AUFPR10	Jajo	″	85±8.66^{c}	21±0.009^{bc}	1.36±0.72^{a}	88	HE
(+) Control	-	″	-	-	1.55±0.04^{a}		
AUGPR11	Hawi	Grass pea	-	-	0.09±0.02^{a}	36	LE
AUGPR12	Amino	″	67±8.54^{b}	47±0.006^{c}	0.16±0.02^{bc}	64	E
AUGPR13	Adadi	″	90±7.21^{b}	62±0.002^{d}	0.20±0.01^{cd}	81	HE
AUGPR14	Segeda	″	135±18.87^{c}	86±0.006^{e}	0.21±0.02^{cd}	84	HE
AUGPR15	Folle	″	24±4.16^{a}	35±0.002^{b}	0.11±0.01^{ab}	44	LE
(+) Control		″	-	-	0.25±0.29^{d}		
AULR16	Adadi	lentil	30±7.64^{b}	15±0.002^{b}	0.06±0.010^{a}	55	E
AULR17	Hawi	″	18±4.04^{bc}	2±0.001^{a}	0.07±0.02^{a}	64	E
AULR18	Folle	″	55±4.58^{d}	17±0.003^{b}	0.1±0.01^{a}	91	HE
AULR19	Amino	″	16±3.28^{ab}	2±0.001^{a}	0.05±0.01^{a}	45	LE
AULR20	Segeda	″	18±4.73^{bc}	6±0.006^{bc}	0.06±0.11^{a}	55	E
(+) Control	-	″	-	-	0.11±0.01^{a}	-	-

Table 4. Morphological and cultural characteristics of selected isolates from faba bean, field pea, grass pea and lentil.

Isolates	Collection site	Host of isolates	Colony diameter	Colony morphology	Mean generation time (MGT) (h)
AUFBR2	Benyo	Faba bean	4	LM, translucent	1.3
AUFBR3	Jajo	Faba bean	4.5	LW, transparent	2.6
AUFBR5	Daloda	Faba bean	6	LW, transparent	1.7
AUFPR6	Kulso	Field pea	3	LM, translucent	3.3
AUFPR7	Dalota	Field pea	2.5	LW, transparent	2.9
AUFPR10	Jajo	Field pea	4.1	LM, translucent	4
AUGPR12	Amino	Grass pea	2.5	LM, translucent	3.9
AUGPR13	Adadi	Grass pea	5.1	LM, translucent	2.2
AUGPR14	Segeda	Grass pea	3.3	LM, translucent	2.8
AULR16	Adadi	Lentil	3	LW, transparent	4.3
AULR17	Hawi	Lentil	3.3	LM, translucent	1.9
AULR18	Fole	Lentil	4	LM, translucent	1.0

NS=north Shoa, LM= Large mucoid, LW= Large watery.

AUFPR6 (field pea), AUGPR12, AUGPR13, AUGPR14(grass pea) and AULR18 (lentil) were able to grow at pH 4, in contrast with Keneni et al. (2010). Keneni et al. (2010) showed that faba bean rhizobia from Wollo, Northern Ethiopia, could not tolerate pH 4. Isolates AUFBR5 (faba bean), AUGPR13 (grass pea) and AUGPR14 (grass pea) showed a wide range of pH tolerance (pH 4 to12).

The isolates developed within a varied range of temperature 5 to 45°C (Table 5). All the isolates (100%)

Table 5. Physiological properties of Isolates from faba bean, field pea, grass pea, and lentil.

Isolates	Host	Range of salt (%)	Range of pH	Range of T (°C)
AUFBR2	Faba bean	1-10	4.5-12	5-40
AUFBR3	Faba bean	1-3	4.5-9	5-45
AUFBR5	Faba bean	1-6	4-12	10-35
AUFPR6	Field pea	1-8	4-9.5	15-35
AUFPR7	Field pea	1-7	5-12	5-35
AUFPR10	Field pea	1- 2	4.5-11	15-40
AUGPR12	Grass pea	1-3	4-10.5	5-45
AUGPR13	Grass pea	1-10	4-12	10-40
AUGPR14	Grass pea	1-10	4-12	5-45
AULR16	Lentil	1-7	5-11	15-35
AULR17	Lentil	1-4	4.5-9.5	15-40
AULR18	Lentil	1-9	4-11	5-45

grew well between 15 and 35°C as reported in (Belay (2006), Amsalu (2007), Tesfaye (2008) and some isolates were found to tolerate 5°C of temperature. This result correlated with (Belay (2006) and Tesfaye (2008) from faba bean isolates, Fano (2010) on field pea and Adal (2009) from grass pea. They also reported that the tolerance of some *R. leguminosarum biovar viciae* isolates at 4, 5, and 10°C.

In this study, isolates AUFBR3 (from faba bean), AUGPR12, AUGPR14 (from grass pea) and AULR18 (from lentil) without a field pea isolates were tolerated with high temperature of 45°C as described by Adal (2009) on grass pea rhizobia in contrast with earlier works on cool season food legumes (Gebremariam, 2007) on faba bean and (Amsalu, 2007; Fano, 2010) on field pea rhizobia (Table 5).

Cross-inoculation and symbiotic effectiveness test

The cross-inoculation of hachallu and wolki varieties of faba bean (*Vicia faba*) with isolates from viceae variety hosts were determined. The ability to form nodules and enough nitrogen of the isolates were evaluated on the basis of nodule number, nodule dry weight and shoot dry weight as described by Brockwel (1998). All of the rhizobial isolates from faba bean, field pea, grass pea, and lentil were successfully cross inoculated and formed nodules on two varieties of Faba bean (*Vicia faba*). The result correlated with the work of McVicar et al. (2005) who reported that *R. leguminosarum bv. viciae* have broad host range than cross-nodulate field pea, lentils, grass pea and faba bean.

Nodules number ranged between 45 to 131 and 41to122 nodules/plant on Hachallu and wolki varieties respectively. The highest number of nodule/ plant (131) was recorded on Hachallu variety inoculated with isolate AUGPR14 (grass pea) and 122 nodules/plant on wolki variety inoculated with isolate AUFBR5 (faba bean). The least number of nodules (45 nodules/pl) was recorded from plants inoculated with isolate AUFPR6 (field pea) from Hachallu variety and 41 nodules/plant from wolki variety inoculated with isolate AUGPR13 (grass pea) (Table 6).

The finding showed that strains isolated from vicieae tribe's cross-inoculation group were able to form nodules with faba bean plant but, the number, color, and shape of nodule varied according to the strain inoculated (Laguerre et al., 2003). Nodules produced by strain on one legumes host may have no resemblance to nodules produced by the same strain on another host in the same cross-inoculation group (Mahajan and Gupta, 2009) (Table 6).

The nodule dry weight was ranged between 0.091 to 0.043 g, and plant inoculated with isolates AUGPR12 (grass pea) and AULR1 (lentil) on wolki variety whereas, 0.017 to 0.001gm/ plant by isolates AUFBR2 and AUFBR3 (faba bean) on Hachallu variety accordingly (Table 6). There was a discrepancy in nodule number and weight among the inoculated plants at P<0.01.

Maximum mean shoot dry mass (4.04g/plant) was recorded by isolate AUGPR13 (grass pea) on Hachallu variety and 3.11 g/plant with isolate AUFBR5 (faba bean) on wolki variety. The minimum shoot dry mass was recorded by plant inoculated with isolates AUFBR5 (faba bean) and AULR17 (lentil) (2.60g/Pl) on Hachallun and 1.74 g/pl by isolate AULR17 (lentil) on wolki variety. This result supported by previous findings of Talukder et al. (2008) in such a way that shoot dry weight was influenced by rhizobium inoculation. In this experiment, a significant difference in shoot dry weight among the isolates from cross inoculated hosts was recorded at p<0.01(Table 6).

The relative symbiotic effectiveness among the test isolates from heterogeneous cross-inoculation hosts were evaluated on the basis of the shoot dry matter accumulation by the infected host plants in reference to the nitrogen-fertilized control (Purcino et al., 2000) (Table7). From faba bean, isolate AUFBR2 was highly

Table 6. Comparative evaluation of symbiotic effectiveness of the modulated isolates with both Hachallu and Wolki varieties of faba bean.

Isolates	Host of isolate	NN/pl		NDW/pl (g)		SDW/pl (g)		% SE		Rate	
		HV	WV	HV	WV	HV	WV	HV	WV	HV	WV
AUFBR2	Faba bean	112^{e}	106^{f-h}	0.001^{b-e}	0.045^{b}	3.32^{a-d}	2.63^{a}	82	99	HE	HE
AUFBR3	Faba bean	65^{bc}	103^{e-h}	0.017^{b-f}	0.074^{c-e}	3.14^{a-d}	2.98^{a}	77	112	E	HE
AUFBR5	Faba bean	112^{e}	122^{h}	0.004^{a-c}	0.058^{b-d}	2.60^{a-d}	3.11^{bc}	64	117	E	HE
(+) control	-	-	-	-	-	4.06^{d}	2.66^{a}	-	-	-	-
(-) control	-	-	-	-	-	1.58^{ab}	0.72^{a}	-		-	-
AUFPR6	Field pea	45^{b}	91^{d-g}	0.002^{ab}	0.068^{b-e}	3.25^{a-d}	2.08^{a}	89	52	HE	E
AUFPR7	Field pea	73^{b-d}	110^{gh}	0.012^{a-d}	0.074^{c-e}	2.91^{a-d}	2.94^{a}	81	115	HE	HE
AUFPR10	Field pea	67^{bc}	87^{d-g}	0.016^{a-e}	0.065^{b-e}	3.09^{a-d}	2.65^{a}	85	104	HE	HE
(+) control	-	-	-	-	-	3.61^{b-d}	2.55^{a}	-	-	-	-
(-) control	-	-	-	-	-	1.68^{a-c}	1.03^{a}	-	-	-	-
AUGPR12	Grass pea	105^{de}	90^{d-g}	0.008^{b-f}	0.091^{e}	3.36^{a-d}	2.90^{b}	82	94	HE	HE
AUGPR13	Grass pea	70^{b-d}	41^{b}	0.005^{d-f}	0.053^{bc}	4.04^{d}	2.13^{a}	98	69	HE	E
AUGPR14	Grass pea	131^{e}	46^{bc}	0.015^{ef}	0.058^{b-d}	3.67^{b-d}	2.71^{a}	89	87	HE	HE
(+) control	-	-	-	-	-	4.11^{d}	3.10^{bc}	-	-	-	-
(-) control	-	-	-	-	-	1.57^{ab}	1.07^{a}	-	-	-	-
AULR16	Lentil	60^{b}	63^{b-d}	0.010^{b-f}	0.043^{b}	2.82^{a-d}	2.50^{a}	74	92	E	HE
AULR17	Lentil	54^{b}	72^{c-e}	0.014^{c-f}	0.086^{e}	2.60^{a-d}	1.74^{a}	68	64	E	E
AULR18	Lentil	103^{c-e}	76^{c-f}	0.003^{f}	0.081^{de}	2.77^{a-d}	2.64^{a}	72	97	E	HE
(+) control	-	-	-	-	-	3.82^{cd}	2.73^{a}	-	-	-	-
(-) control	-	-	-	-	-	1.31^{a}	0.83^{a}		-	-	-

HV=hachallu variety, WV=wolki variety of faba bean, Pl=plant, NN=nodule number, NDW=nodule dry weight, SDW=shoot dry weight, SE=symbiotic effectiveness

Table 7. The relative symbiotic effectiveness of the isolates from heterogeneous cross-inoculation host on two varieties of faba bean.

Host of isolate	Hachallu variety of faba bean		Wolki variety of faba bean	
	Effective	Highly effective	Effective	Highly effective
Faba bean	AUFBR3, AUFBR5	AUFBR2	-	AUFBR2, AUFBR3 and AUFBR5
Total	2	1	-	3
Percentage (%)	66.7	33.3	-	100
Field pea	-	AUFPR6, AUFPR7, AUFPR10	AUFPR6	AUFPR7, AUFPR10
Total		3	1	2
Percentage (%)		100	33.3	66.7
Grass pea	-	AUGPR12, AUGPR13, AUGPR14	AUGPR13	AUGPR12, AUGPR14
Total		3	1	2
Percentage (%)		100	33.3	66.7
Lentil	AULR16, AULR17, AULR18	-	AULR17	AAULR16, AAULR18
Total	3	-	1	2
Percentage (%)	100	-	33.3	66.7

effective; isolates AUFBR3 and AUFBR5 were effective on Hachallu variety whereas, all isolates were highly effective on wolki variety. All isolates of Field pea were highly effective on Hachallu variety whereas, isolates AUFPR7 and AUFPR10 were highly effective and isolate AUFPR6 was effective on Wolki variety.

Similarly, all isolates of grass pea were highly effective on Hachallu variety, and isolate AUGPR12 and AUGPR14 were highly effective with 1isolate (AUGPR13) effective on wolki variety. All isolates of lentil were effective on Hachallu variety and 2 isolates (AULR16 and AULR18) and 1 isolate (AULR16) were highly effective and effective on wolki variety respectively (Table 7). This result indicated that *R. leguminosarum* bv.*viciae* isolated

Table 8. Summary of physiological and symbiotic properties of Isolates from faba bean, field pea, grass pea and lentil.

Isolates	Host	Range of salt (%)	Range of pH	Range of T (°C)	% SE		Rate	
					HV	WV	HV	WV
AUFBR2	Faba bean	1-10	4.5-12	5-40	82	99	HE	HE
AUFBR3	Faba bean	1-3	4.5-9	5-45	77	112	E	HE
AUFBR5	Faba bean	1-6	4-12	10-35	64	117	E	HE
AUFPR6	Field pea	1-8	4-9.5	15-35	89	52	HE	E
AUFPR7	Field pea	1-7	5-12	5-35	81	115	HE	HE
AUFPR10	Field pea	1- 2	4.5-11	15-40	85	104	HE	HE
AUGPR12	Grass pea	1-3	4-10.5	5-45	82	94	HE	HE
AUGPR13	Grass pea	1-10	4-12	10-40	98	69	HE	E
AUGPR14	Grass pea	1-10	4-12	5-45	89	87	HE	HE
AULR16	Lentil	1-7	5-11	15-35	74	92	E	HE
AULR17	Lentil	1-4	4.5-9.5	15-40	68	64	E	E
AULR18	Lentil	1-9	4-11	5-45	72	97	E	HE

T=temperature, HV=hachallu variety of faba bean, WV=wolki variety of faba bean; SE=symbiotic effectiveness, HE=highly effective, E=effective.

from different host legumes showed variation in symbiotic effectiveness on faba bean varieties. The result is similar with that of Shimeket et al*.* (2008) who reported variation in symbiotic effectiveness from a population of *R. leguminosarum* bv.*viciae* on vetch from different soils*.* Laguerre et al. (2003) reported that specific symbiotic plasmid-encoded genes showed variation in competition for modulating of faba beans and nitrogen-fixing effectiveness.

Generally, isolates of AUFBR2 (faba bean) and AUGPR14 (grass pea) were highly effective in both hachallu and wolki varieties, and showed tolerance wide range of salt, pH, and temperature than their cross-inoculation group of *R. leguminosarum var viciae* (Table 8). These remarkable features of isolates are very important for screen, and it’s shown to be competitive for future inoculants legumes production under extreme environmental conditions at the field trials in the soil ecosystem.

Conclusion

The present study shows the physiological and symbiotic diversity of *R. leguminosarum bv.viciae* isolates of faba bean, field pea, grass pea, and lentil. Some of the isolates showed tolerant to a wider range of salt, pH, and temperature. Twelve isolates of faba bean, field pea, grass pea, and lentil were cross inoculated and induced nodules on faba bean host. Isolates from different cross-inoculated were effective irrespective of their host from which they isolate. The symbiotic effectiveness of isolates showed a significant difference on Hachallu variety (64 to 98% SE) and Wolki variety (52 to 117% SE). Isolates of AUFBR2 (faba bean) and AUGPR14 (grass pea) were best matched with both hachallu and wolki varieties followed by isolates AUFPR2 (field pea) and AUGPR3 (grass pea). This matching of rhizobial isolates to host legume is one of the most important factors in maximizing productivity and full expression of N_2 fixation in stressed environmental condition.

RECOMMENDATIONS

Isolates of AUFBR2 (faba bean), isolate AUGPR14 (grass pea), AUGPR13 (grass pea) and AUFPR7 (field pea) must be used to develop inoculants as they are ecologically competitive. Screening isolates genetically using molecular techniques of REP/PCR or RFLP/PCR to evaluate their effectiveness under different environmental conditions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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