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Effect of Additive Series Intercropping Kidney Bean (*Phaseolus vulgaris* L.) with some Aromatic Plants on *Tetranychus urticae* Koch. (Acari: Tetranychidae) Population †

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Abstract: Intercropping (a farming practice involving two or more crop species, growing together and coexisting for a time) is one of the important cultural practices in pest management and is based on the principle of reducing insect pests by increasing the diversity of an ecosystem. To evaluate the impact of additive series intercropping kidney bean with some aromatic plants on different biological stages (eggs, immatures, and adults) of two-spotted spider mite (TSSM) the experiment was carried out in a randomized complete blocks design (RCBD) with five treatments included kidney bean pure stand, kidney bean+Coriander (100+50) kidney bean+ajwain, (100+50) kidney bean+basil, (100+50) and (100+50) kidney bean+dill and three replicates in the cropping year 2020-21, Lorestan province, Iran. The results showed that the intercropping system significantly influencesd on the different life stages of TSSM, e.g., the minimum and the maximum number of eggs, immatures, and adults of TSSM were found in kidney bean+basil (3.880, 4.103, 1.113) and pure bean (7.783, 7.107, 2.33) treatments, respectively. Also, the correlation test results showed a significant negative relationship between the number of eegs, immatures, and adults with kidney bean yield. Moreover, the highest yield (2756 kg/ha^{-1}) and Land Equivalent Ratio (1.43) were recorded in kidney bean+basil. Finally, can be expressed intercropping kidney beans with some aromatic plants proved to be an eco-friendly strategy in the reduction of TSSM population in kidney bean farmland.

Keywords: sustainable agriculture; polyculture; egg; immature; adult; biological control

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1. Introduction

Two-spotted spider mite (TSSM), *Tetranychus urticae* Koch (Acari: Tetranychidae), is one of the most common and severe pests in greenhouses and fields. It is a polyphagous species, with high genomic adaptations, which facilitates its feeding and reproduction on more than 1100 plant species belonging to 140 plant families [1] (pp. 487-492).

Kidney bean (*Phaseolus vulgaris* L.) is a major legume that is consumed worldwide for its edible seeds and pods, and because of its high protein content (20–25%), complex carbohydrates (50–60%), a good source of vitamins, minerals and poly-unsaturated fatty acids, it could be considered as an essential component of human diet [2] (pp. 351-353).

Legumes are highly vulnerable to insect pests; several insect pests including bean flies, aphids, thrips, leafhopper, whiteflies, leaf beetles, pod borers, pod bugs and bruchid beetles cause significant damage to legumes in the field or in storage [3] (pp. 426-431).

Accordingly, the TSSM, is a cosmopoliatan and key pest of kidney bean plants [4] (pp. 319-335).

TSSM control relies on synthetic pesticides mostly despite their hazardous side effects [5] (pp. 715-720), which might induce pest outbreaks [6] (pp. 339-354). Besides increasing the production cost of production, pesticides have negative effects on the environment and human health, attributed to high chemical residues [7] (pp. 55-59).

One of the ecofriendly novel method of managing pests is intercropping [8] (pp. 24-30) (defined as growing more than one type of crops in close proximity simultaneously), where plants coexist at least throughout the prominent part of their cycle [9] (pp. 1086-1092). Today, intercropping is considered as an essential component of crop production systems in developing countries [10] (pp. 51-66). It can promote numerous benefits to cropping systems through increasing total yield and land use efficiency [11] (pp. 249-256), improving biodiversity and yield stability of cropping systems [12] (pp. 106-113), enhancing light, water, and nutrients use [13] (pp. 95-99), reducing pest pressure [14] (pp. 509-520), increasing soil fertility and conservation of natural enemy fauna [15] (p. 14).

Medicinal herbs which are continuously releasing volatile organic compounds (VOCs) play an important role in tritrophic interactions of plant–pest–natural enemies [16] (pp. 67-90). Essential oils of Amaryllidaceae, Lamiaceae, Meliaceae, Rutaceae, and Solanaceae plant families offer some acaricidal and/or repellent characteristics against two-spotted spider mite [17] (pp. 361-386).

Medicinal plants are considered as sources of health products, essential oils, and other natural aroma chemicals in the national and international markets [18] (pp. 78-83). Aromatic plants (AP), produce high amounts of volatile secondary metabolites, thus they are potential candidates for testing the intercropping designs aimed at interfering or masking host plant odors to disturb host selection by insect pests. Several studies have emphasized the use of medicinal plants such as cornflower (*Centaurea cyanus* (L.) [19] (pp. 179-187), summer savory (*Satureja hortensis* L.) [20] (pp. 13-18), sweet basil (*Ocimum citriodorum* Vis.) [21] (pp. 537-547), chili pepper (*Capsicum frutescens* Linn.) [22] (pp. 63-66) and saffron (*Crocus sativus* L) [23] (pp. 3060-3065) in intercropping systems.

Agroecologists have suspected that increasing plant diversity within agricultural fields changes food web structure and thereby reduces herbivore abundance and crop damage [24] (pp. 9-21). Determining the effects of plant diversification on pest control and determining the scale at which plant diversity affects pest control remain key challenges for sustainable agriculture [25] (pp. 1715-1727). In their meta-analysis based on articles published between 1998 and 2008, [24] (pp. 9-21) showed that plant diversification increases herbivore suppression and the abundance of natural enemies and decreases crop damage.

Another result of mixed cropping is that crops grown simultaneously enhance the abundance of predators and parasites, which in turn prevent the pest population build-up, thus minimizing the need of using expensive and hazardous chemicals [26] (pp. 396-410). Recently, some studies have demonstrated that the use of intercropped repellent plants or trap plants can be effective alternative methods to reduce pest pressure on the primary crop[27] (pp. 273-285).

Despite wide host ranges, two-spotted spider mite populations were shown to be influenced by intercropping practice. The use of garlic, other Amaryllidaceae plant, as intercropping plant reduced TSSM populations by 65% in strawberry leaves in a filed study [28] (pp. 311-321). The TSSM populations were reduced when Amaryllidaceae plants were intercropped in strawberries (*Fragaria ananassa* Duchesne)[29] (pp. 178-182).

Regarding the adverse side effects of using pesticides on human health, food and environment, our hypothesis is that, aromatic plants reduce TSSM populations on kidney bean leaves through an intercropping system. Accordingly, the current study aimed to evaluate the impact of additive intercropping kidney bean with some aromatic plants on the different life stage population of T. urticae.

2. Materials and Methods

2.1. Experimental site

Field experiments were conducted at the Malekabad Research Farm (Alashtar, Lorestan province, Iran ;33°83' N latitude, 48°24' E longitude, 1620 m above mean sea level), in 2020 under irrigated conditions. The experimental site has an average annual precipitation of 440.30 mm, which classified as a semi-arid climate [30]. The annual mean temperature of the region was recorded as 12.7°C, which was 21.67°C during the experiment. The study was conducted at soil conditions with pH=7.5 and low salt content. All treatments received the same fertilization regime depnding soil analysis results.

2.2. Design of Experiment

The experiment was carried out in a randomized complete blocks design (RCBD) with five treatments and three replicates. The treatments included kidney bean pure crop (*Phaseoulus vulgaris* L. c.v. Dadfar), (100+50) kidney bean+Coriander (*Coriander sativum* L.), (100+50) kidney bean+Ajwain (*Carum copticum* L.), (100+50) kidney bean+Basil (*Ocimum basilicum* L.), and (100+50) kidney bean+Dill (*Anethum graveolens* L.) (the pattern of intercropping was as additive series). Local cultivars of additive intercropped plants were used in the study. The size of plots was 3×2.5 (Length × Width) and the density of kidney bean was 20 plants/m^2 and coriander, ajwain, basil, and dill were sown between kidney bean rows (one row in between) with 8, 6, 10, and 20 cm distance from each other on the stacks, respectively. After sterilization by Rosalaxyl 72%, WP (Ridomil-Mancozeb) for 15 min, kidney bean, and other intercropped seeds were planted at the stack side (width ca. 50 cm) on 24th May 2020. Kidney bean seeds were sown in the depth of 5 cm, and four additives intercropped plant seeds were sown in 1 cm soil depth. Irrigation and weed control were carried out as required. After bean pod ripening, yield per hectare was calculated, and Land Equivalent Ratio (LER) regarding seed yield evaluated according to equation 1.

$$\text{LER} = \frac{\text{Yb}}{\text{Ya}} \quad (1)$$

Where Yb is the kidney bean yield in intercropping and Ya is the yield of kidney bean in pure culture treatment.

LER < 1, meaning less than expected (adverse effect)

LER = 1, meaning the same as monoculture

LER > l, meaning more than expected (favorable effect)

According to equation 1, if LER is greater than one, the intercropping system work but if LER is less than one, the pure cultivation will be more profitable.

2.3. Sampling method

Samples were taken once the TSSM emergence in the plots and there were six sampling dates on a weekly basis. Because of marginal effect, no samples were taken from lines one and six and 50 cm up and down the plots. Kidney bean leaf was selected as the sampling unit and sampling was conducted by randomly selecting six plants and four leaves per plant at four geographical directions from the middle in the lower and upper surface in each sampling date. Different life stages of (egg, immature, and adult) *T. urticae* were examined under a digital loop (400x magnification) after transfer to the laboratory.

2.4. Statistical analysis

The analysis of variance assumptions were checked by normality test. If assumptions were met, depended variables were compared by an analysis of variance (ANOVA) and means were compared using Tukey's multiple range test., Moreover, the relationships between independent variables were assessed by the correlation coefficient.

3. Results

3.1. Effect of intercropping on different life stages of Tetranychus urticae

Statistical analysis revealed that the population densities of TSSM immatures (F= 95.61, df= 4, 8, P <0.01), adults(F= 17.09, df= 4, 8, P <0.01) and eggs (F= 19.55, df= 4, 8, P <0.01) were significantly influenced by intercropping treatments. As well, the mean comparison showed the highest number of eggs, immatures, and adults (7.783, 7.107, and 2.330 respectively) were found in pure kidney bean while, the lowest values were recorded in (100+50) kidney bean+basil treatment (3.880, 4.103, and 1.113 respectively) (Table 1). However, among the intercropped treatments, after (100+50) kidney bean+basil treatment, (100+50) kidney bean+ajwain (6.027), and (100+50) kidney bean+dill (5.743) treatments, had the lower number of eggs. Also, statistical analysis about immatures of TSSM revealed that there were no significant differences between (100+50) kidney bean+basil, (100+50) kidney bean+ajwain, and (100+50) kidney bean+dill (Table 1). Moreover, adult densities differed among (100+50) kidney bean+coriander and (100+50) kidney bean+ajwain treatments with (100+50) kidney bean+basil treatments. Notably, all intercropping treatments had a lower number of eggs, immatures, and adults than kidney bean monoculture.

3.2. Kidney bean yield and Land Equivalent Ratio (LER)

Analysis of variance showed that kidney bean yield (F= 17.87, df= 4, 8, P <0.01) and LER (F= 16.54, df= 4, 8, P <0.01) significantly influenced by intercropping (Table 1). Accordingly, mean comparisons concerning kidney bean yield and LER revealed a significant difference between pure and intercropped treatments. For instance, kidney bean pure had a significantly lower performance (1926 kg/ha^{-1}), than (100+50) kidney bean+basil treatment (2756 kg/ha^{-1}).

In the current study, the LER of all intercropping treatments was more than 1, indicating an advantage of intercropping compared to the monoculture of kidney bean (Table 1). Among intercropped treatments, a (100+50) kidney bean+basil treatment had a higher value (1.433) rather than other intercropping treatments. As shown in Table 1, there were no significant diffrences between (100+50) kidney bean+Ajwain, (100+50) kidney bean+Coriander and (100+50) kidney bean+Dill

3.3. The relationship between the different life stages of TSSM and kidney bean yield

The correlation test results showed a significant negative relationship between the number of eggs (Correlation coefficient = -0.889, P-value < 0.05), immatures (Correlation coefficient = - 0.829, P-value < 0.05) and adults (Correlation Coefficient = -0.776, P-value < 0.05) with kidney bean yield, which means treatments with high yield should have a fewer number of *T. urticae* eggs, immatures, and adults. For example, kidney bean+basil (100+50) with 2.756 t/ ha have fewer eggs, immatures, and adults (3.88, 4.103, and 1.113, respectively).

Table 1. Mean ± SE number of *Tetranychus urticae* life stages, yield (per hectare) and LER (Land equivalent ratio) at different intercropping treatments (Tukey's 1%).

Treatments	Eggs	Immatures	Adults	Yield	LER
Kidney bean pure	7.783±0.158^{a}	7.107±0.477^{a}	2.33±0.076^{a}	1926±54.20^{c}	1.0±0 ^{c}
Kidney bean+Coriander (100+50)	7.080±0.141^{a}	6.183±0.172^{ab}	2.217±0.094^{ab}	2280±1.09^{bc}	1.188±0.068^{bc}
Kidney bean+Ajwain (100+50)	6.027±0.174^{b}	5.553±0.454^{abc}	1.907±0.157^{ab}	2301±69.24^{abc}	1.197±0.049^{bc}
Kidney bean+Basil (100+50)	3.88±0.058^{c}	4.103±0.016^{c}	1.113±0.032^{C}	2756±57.18^{a}	1.424±0.065^{a}
Kidney bean+Dill (100+50)	5.743±0.135^{b}	4.99±0.221^{bc}	1.473±0.153^{bC}	2445±66.47^{ab}	1.270±0.024^{ab}

[2] Different lowercase letter in each column indicates significant differences at 0.05

4. Discussion

The results of our study showed that aromatic plants are not a preferred hosts for TSSM development and reproduction. Accordingly, the densities of *T. urticae* life stages were lower on all intercropping treatments, and significant reductions were found in (100+50) kidney bean+basil treatment.

It is widely known that diversifying agriculture systems (e.g. through intercropping) prevents pest outbreaks and increases the sustainability of agroecosystems [31] (pp. 39-44). Intercropping increases the diversity of plant species in the cropping systems and is considered to make the systems more resilient against environmental perturbations, thus enhancing food security [32] (pp. 238-253).

Two main hypothesis for the effect of intercropping are resource concentration hypothesis and the natural enemies' hypothesis. The first one emphasized decreasing pest availability and retention in the preferred crop [33] (pp. 484). Accroding to this hypothesis, finding a host plant may be more difficult when non-host plants surround it and interfere with the host plant selection process. However, because the plant species used for intercropping differ, this approach outcomes vary regarding the intercropped plant combinations and pest species. It has been discussed how both chemical and visual stimuli affect the behavior of pests during the period before accepting a plant as a suitable host (theory based on appropriate/inappropriate landings) [34] (pp. 91-102).

The underlying mechanism most likely involves the repellent chemicals hypothesis [35] (pp. 159-167), in which the volatile organic compounds (VOCs) rleased from aromatic plants interfere with the ability of a herbivore to find a host plant, feed, migrate and breed, thus preventing pest population build-up [36] (pp. 183-195). Indeed, VOCs have insecticidal, antifeedant, and repellent effects on herbivorous arthropods [37] (pp. 3590-3601). Several studies have documented the repellent role of volatiles in host plant selection, feeding and mating by phytophagous insects [38] (pp. 7-11).

The obtained data are in line with those previously reported by [39] (pp, 215-230) which studied the effect of intercropping of aromatic plants on the population of three main pests and their associated predators with three bean varieties at Fayoum and Gharbia Governorates, Egypt. They proved that Mint, Fennel and Black cumin plants played a critical role to reduce the population of *T. urticae* on *P. vulgaris* in Gharbia and Fayom Governorates. Our results are in accordance with obtained in another previous study in which intercropping garlic reduced the number of TSSM mobile forms and eggs in strawberry when garlic was intercropped in the field [28] (pp. 311-331). Aromatic plants may also make strawberry leaf less preferred by TSSM, since diallyl disulfide, which has an antifeedant emanates from garlic [40] (pp. 537-543).

The other advantage of intercropping is the more efficient utilization of the available resources and the increased productivity compared with monoculture cropping [41] (pp. 132-139). Yield increase occurs because environmental factors such as light, water, and nutrients are more efficiently absorbed and converted to crop biomass by the intercrop over time and space. This could be assigned to differences in competitive ability for resources among the involved crops, which exploits the variations of the mixed crops such as canopy development rates, final canopy size (width and height), a photosynthetic adaptation of canopies to irradiation conditions, and root taking depth [42] (pp. 17-29).

5. Conclussion

Our results showed that *T. urticae* colonization and population buildup on kidney bean host plant negatively influenced by aromatic plant volatile in a intercropped system, so it can be said intercropping with scented plants which are known for their essential oils and are widely implemented in agricultural systems for their insecticidal, antifeedant, and repellent effects may beemployed as an integrated pest management tool of kidney bean. Also, the addition of plant resources may increase the survival, potential fertility, behavior, and effects of natural enemies. Therefore, increasing plant diversity and habitat manipulation for natural enemies reduces the pest pressure and consequently pesticide consumptions in farming. However, on the other side, mixed vegetation mightly reduces the searching efficiency of natural enemies and destabilize the predator-prey interactions. Finally, intercropping kidney bean with aromatic plants can increase crop yield and decrease the TSSM population rather than monoculture. Thus, most ecologists advocate intercropping as an integrated pest management (IPM) practice to control pests.

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